PROPOFOL: ANALYTICAL TECHNIQUES AND APPLIED PHARMACOKINETICS

BY

WONG KWOK KONG

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ABSTRACT

Propofol is a commercially available intravenous anaesthetic agent. The main feature of propofol is rapid recovery from anaesthesia, whether given as a bolus for induction of anaesthesia or by infusion to maintain anaesthesia.

The aim of this thesis was to develop analytical techniques for measuring the concentration of propofol. It details the assay of propofol in blood samples using high-pressure liquid chromatography (HPLC) with fluorescence detection, and propofol protein binding studies using equilibrium dialysis. Examples are given of clinical applications of these techniques.

The reversed phase HPLC method used was simple, sensitive, and reliable. The assay involved a single extraction of the drug and internal standard, thymol, from blood buffered with 0.1 M sodium dihydrogen phosphate buffer into cyclohexane. The organic extract, basified with tetramethylammonium hydroxide, was evaporated to dryness at 37°C under nitrogen. The residue was redissolved in 80 μl acetonitrile and an aliquot of the concentrate was injected into a C18 reversed-phase column. The mobile phase

consisted of 66% (v/v) acetonitrile in Milli-Q water containing 1% v/v acetic acid and was eluted at 1.7 ml/min. The components of the column effluent was monitored by a fluorometric detector with excitation and emission wavelengths set at 276 nm and 310 nm, respectively. The main advantage of my method for detecting propofol is that only 0.5 ml of whole blood is required. This facilitates pharmacokinetic studies of propofol in children. The calibration graphs were linear over the range 2-3000 ng/ml with coefficients of variation ranging from 0.38 to 7.86%, while the limit of detection was approximately 2 ng/ml. The intraassay coefficient of variation of propofol in whole blood was 6.24% at 100 ng/ml and 6.10% at 500 ng/ml, while the interassay coefficient of variation was 3.18% at 100 ng/ml and 1.81% at 500 ng/ml. The extraction recovery results obtained for propofol in whole blood are 104% at 100 ng /ml and 99% at 500 ng/ml. Stability of propofol in whole blood is one month. The results of propofol assay in plasma are similar to propofol assay in whole blood.

For the protein binding assay, equilibrium dialysis was performed using a Spectrum equilibrium Dialyser. Drug-containing plasma samples or protein solutions (1 ml) were dialysed against drug-free Sorensen's phosphate buffer (1 ml; pH 7.4) in teflon dialysis chambers separated by Spectra/Por dialysis membrane. The effect of drug concentration on protein binding was determined within the

range of 0.25-3.00 μg/ml of propofol. The teflon cells rotate at 15 rpm at 37°C. The optimum dialysis time was 240 min. Propofol concentration in samples were assayed by HPLC as described above. The intraassay coefficient of variation of propofol protein binding was 0.11% (3.65% Free), while the interassay coefficient of variation of propofol protein binding was 0.23% (8.57% Free). The extraction recovery results obtained for propofol in plasma are 96.42% in unheated samples, 90.64% for samples heated at 37°C, and 97.15% samples dialysed at 37°C in a water bath.

One application of my research was the prospective testing in chinese children of a previously published pharmacokinetic model driven algorithm for computer controlled infusion of propofol. The model was revised using an iterative linear least squares regression and minimizing total mean squared prediction error for each patient. The precision of the revised model was 21.08% and bias was 0.19%.

Another application of my research was to investigate protein binding of propofol in different populations because pharmacological response to drugs is influenced by the free fraction of drug. There were no differences in protein binding between young children [< 3 years, 98.77 ± 0.29% (mean ± S.D.)], children (3-12 years, 98.69 ± 0.33%) and adults (98.35 ± 0.29%).

Pregnant patients had increased free propofol, 1.41 ± 0.30% compared with nonpregnant patients, 1.08 ± 0.26% ($p = 0.004$). Free propofol in umbilical plasma 3.40 ± 0.61% was greater than that in maternal (pregnant) plasma ($p = 0.001$). These findings indicate that further simultaneous pharmacodynamic/pharmacokinetic studies would be useful to determine the clinical significance of changes in protein binding.

Acknowledgment

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List of Abbreviations

ACTH	Adrenocorticotrophic hormone
ASA	American Society of Anesthesiologists
ALT	Alanine transaminase
AST	Aspartate transaminase
AUC	The area under the concentration time curve
CBF	Cerebral blood flow
C_{bu}	Concentration in buffer after dialysis
Cl	Total body clearance
C_p	Concentration in plasma after dialysis
C_{TG}	Target concentration
CPP	Cerebral perfusion pressure
C.V.	Coefficient of variance
CVR	Cerebrovascular resistance
ECG	Electrocardiogram
ED_{50}	Effective dose for 50% of population
ED_{95}	Effective dose for 95% of population
EEG	Electroencephalogram
E_TCO_2	End-tidal carbon dioxide
HPLC	High-pressure liquid chromatography
ICP	Intracranial pressure
i.v.	intravenous

kg	kilogram
mg	milligram
ml	millilitre
min	minutes
mol	mole (amount of substance)
MWCO	The molecular weight cut off
ng	nanogram
rpm	Revolutions per minute
S.D.	Standard deviation
$t_{1/2a}$	The early distribution phase half-life
$t_{1/2\beta}$	The elimination phase half-life
$t_{1/2r}$	The terminal phase half-life
T_i	Inspiratory time
T_{tot}	Total ventilatory cycle time
V_c	The apparent volume of distribution in the central compartment
V_d	The apparent volume of distribution during elimination
Vi	Minute volume
V_r	The volume of distribution at distribution equilibrium
Vt	Tidal volume
V_{ss}	The apparent volume of distribution at steady-state
μg	microgram
μl	microlitre

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CHAPTER ONE

INTRODUCTION

Propofol (2,6-diisopropyl phenol) is an intravenous anaesthetic agent which is metabolized rapidly. After anaesthesia with propofol, patient recovery is typically rapid and clear headed. Although the main role of propofol was initially that of a drug to be given as a bolus for induction of anaesthesia, the large total body clearance of propofol also made it suitable for administration by infusion for the maintenance of anaesthesia. The perceived clinical advantages of intravenous anaesthesia with propofol has led to a rapid expansion in both the patient groups in which propofol is being used and the anaesthetic techniques for delivering propofol.

Rational and appropriate dosing regimens for a drug are calculated from the known pharmacokinetics of that drug. The pharmacokinetics of propofol, as for any drug, were first determined in healthy adults. However this information may not be applicable to different patient groups such as the obstetric, neonatal and paediatric populations. Researchers in the Department of Anaesthesia

and Intensive Care at the Prince of Wales Hospital, Chinese University of Hong Kong, were interested in studying anaesthesia with propofol in obstetric and paediatric patients but two potential problems were forseen in determining the pharmacokinetics of propofol in these groups.

Firstly, blood sampling in the paediatric population is difficult and limited ethically by the volume of blood which may be removed. Thus a method must be established for determining propofol concentrations accurately with as small a blood volume as is practical. It was thought that existing methods for the assay of propofol could be further developed and refined.

Secondly, propofol was known to be highly bound to plasma proteins and red blood cells. Although it is accepted generally that only the unbound drug is free to cross cell membranes, be distributed, exert an effect and be metabolized, there was little detailed information on the protein binding of propofol. Such data could be important in the obstetric, neonatal and paediatric patient groups because they have significant alterations in protein and blood composition compared with normal healthy adults.

This thesis reports the development of two analytical methods for measuring propofol: a sensitive assay of total

blood propofol using small blood volumes and a method for determining the bound and unbound fraction of propofol in blood. A summary of the pharmacology of propofol and some examples of the clinical application of these two analytical methods are also presented.

CHAPTER TWO

REVIEW OF

THE PHARMACOLOGY OF PROPOFOL

The commercial preparation 'Diprivan' (Zeneca Pharmaceuticals) is a white, isotonic oil-in-water emulsion for intravenous injection containing 10 mg propofol per ml. The vehicle contains a 1% w/v solution in an aqueous emulsion of 10% soya bean oil, 2.25% glycerol, 1.2% purified egg phosphatide and sodium hydroxide.

1: Chemistry, Structure-Activity relationship

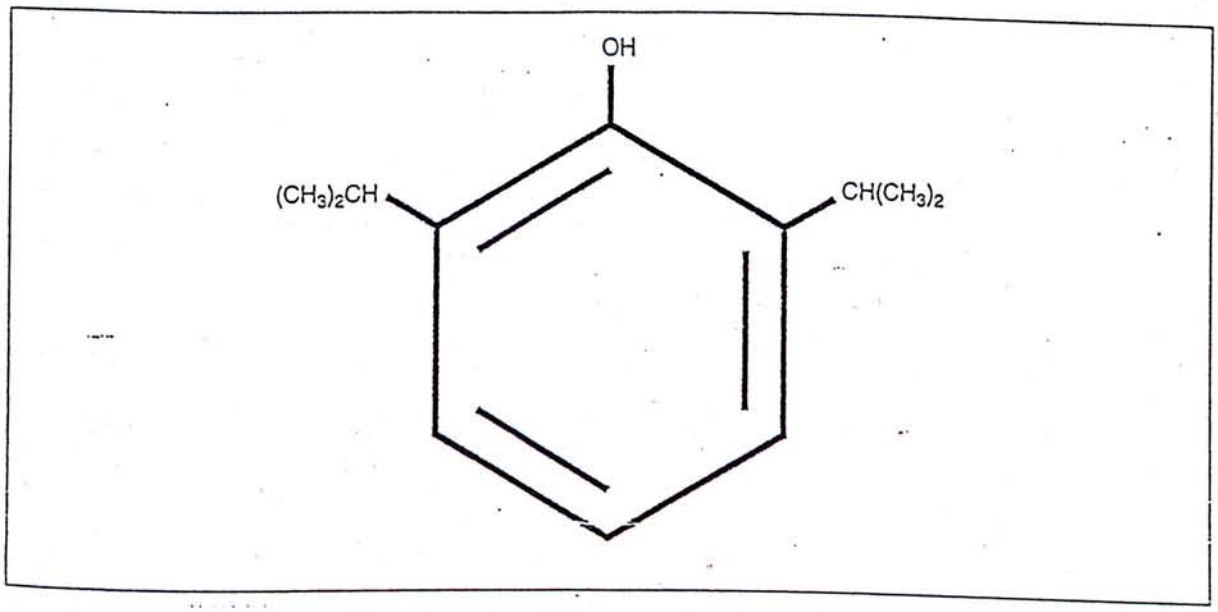

Fig. 2-1: Structural formula of propofol

Propofol (2,6-diisopropylphenol: Fig. 2-1:) represents a new class of intravenous anaesthetic agent, chemically unrelated to barbiturate, steroid or eugenol agents. It is one of a series of sterically hindered phenols that exhibit anaesthetic activity (James *et al*, 1980). It has a molecular weight of 178 daltons and is a colourless liquid at room temperature.

The compound absorbs in the ultraviolet range of the electromagnetic spectrum (λ max 275 nm) and fluoresces at 310 nm with an excitation wavelength of 276 nm. Fluorescence detection with high pressure liquid

chromatography forms the basis of the standard blood concentration assay technique (Plummer 1987).

Substitution at the ortho position of the parent compound, phenol, induces steric hindrance in the adjacent hydroxyl group, modifying the hydrogen bond donor-acceptor properties. This structural change in turn attenuates the protein denaturing effect and confers anaesthetic activity (James *et al.* 1980). Optimal anaesthetic activity is obtained by substitution at the 2 and 6 positions with secondary alkyl groups so that there are a total of six to eight carbon atoms in the substituent chains. Increasing the number of carbon atoms beyond eight leads to a slower induction time, prolonged recovery time, and ultimately loss of anaesthetic action.

2: Pharmacokinetics

The pharmacokinetics of propofol have been evaluated in surgical patients receiving single or repeat bolus doses, and also continuous infusions. Propofol undergoes rapid and extensive distribution, and rapid metabolic clearance. Due to high lipophilicity and extensive tissue binding, the volumes of distribution are very high and the steady state and elimination phase volumes of distribution exceed total body water.

2.1: Distribution

Blood concentration-time curves obtained following single bolus injections show that propofol very rapidly distributes from the circulation into tissues. Estimations of the distribution half-life ($t_{1/2a}$) have varied from 1.8 to 4.7 minutes. Autoradiographic studies in rats demonstrated that propofol appears in the brain within 30 seconds of intravenous administration (Rhodes & Longshaw 1977). Pharmacokinetic modelling of human data indicated a mean blood-brain equilibration half-time of 2.9 minutes (Schüttler *et al.* 1988), which concurs with propofol's rapid onset of action. The apparent volumes of distribution of propofol in the central compartment (V_c), at steady-state (V_{ss}) and during the elimination phase (V_d) are high (13 to 76 L, 171 to 349 L and 209 to 1008 L), reflecting extensive tissue distribution of propofol related to high lipophilicity (Cockshott 1985).

2.2: Elimination

When a subanaesthetic dose of ^{14}C-labelled propofol was given intravenously to 6 male volunteers (mean dose 0.47 mg/kg), 88% of the administered radioactivity was recovered in the urine, whilst less than 2% was excreted in the faeces (Simons *et al.* 1988).

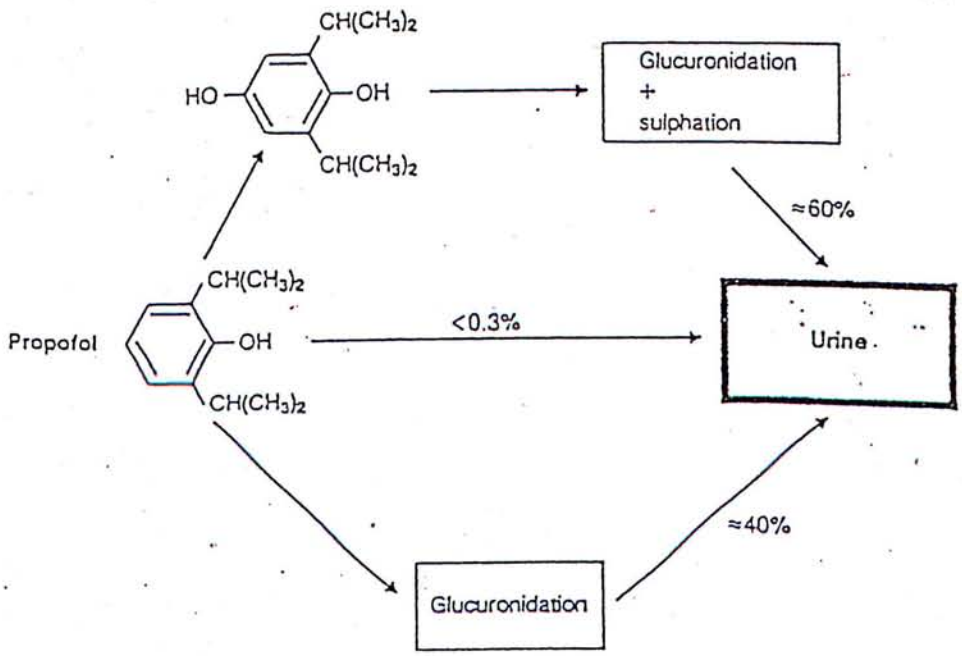

Fig. 2-2: The metabolism and urinary excretion of propofol (after Simons *et at.* 1988)

Analysis of the radioactive material excreted in urine revealed that less than 0.3% was unchanged propofol, whilst approximately 40% was propofol glucuronide and the remainder consisted of the 1-and 4-glucouronide and 4-sulphate conjugates of 2,6-diisopropyl 1,4-quinol (**Fig. 2-2:** The metablolism and urinary excretion of propofol). Metabolism of propofol is rapid; in the above study unchanged propofol accounted for 94% of the radioactive material in blood 2 minutes after injection, but after 30 minutes 81% of the radioactivity was in the form of metabolites. The total clearance of propofol given either as a bolus injection or as an infusion to patients receiving no other anaesthetic agent was between 94 and 139 L/h. A similar range was observed when supplementary anaesthetics were given (108 to 136 L/h). Since normal hepatic blood flow in man has been estimated to be between 66 and 108 L/h (George 1979), these clearance values for propofol suggest that some extrahepatic metabolism occurs (Cockshott 1985), particularly as hepatic blood flow is reduced in anaesthetized patients (Nies *et al.* 1976).

The pharmacokinetics of propofol have been described using both open 2-compartment (Schüttler *et al.* 1985; Simons *et al.* 1988) and open 3-compartment models (Cockshott *et al.* 1987; Gepts *et al.* 1987; Kay *et al.* 1986). In those studies, the concentration time data are best fitted by a 3-compartment model. The $t_{1/2a}$ of 1.8-4.1 minutes is attributed to distribution from blood into

tissues; a $t_{1/2\beta}$ of 25-56 minutes represents metabolic clearance from blood; whilst the terminal elimination phase occurred more slowly with a half-life($t_{1/2r}$) of 184 to 309 minutes following single bolus injections, and of 277 to 403 minutes following continuous infusions (**Table 2-1:** Pharmacokinetic data for propofol obtained surgical patients). Since this terminal elimination phase is probably a consequence of the slow return of propofol from a poorly perfused compartment, this apparent increase in $t_{1/2r}$ following continuous infusion may be due to accumulation of propofol in this compartment.

Table 2-1:

Pharmacokinetic data for propofol obtained in surgical patients

Reference	Number of Patients	Does	Pre-medication	Inhalation agents	Clearance L/h	Volumes of distribution (L) Vdc	Vdss	Vdγ	t1/2α (min)	t1/2β (min)	t1/2γ (min)
Gepts et al, 1987	6/M&F	3 mg/kg/h infusion	Glycopyrroniu bromide	None	113	21	349	1008	3.1	32	403
	6/M&F	6 mg/kg/h infusion			112	16	348	973	3.2	38	386
	6/M&F	9 mg/kg/h infusion			94	13	176	598	2.3	25	277
Cockshott et al, 1987	6/F	2.5 mg/kg bolus	None	N2O	115	41	305	722	2.9	45	284
	6/F		100mcg Fentanyl	N2O	77	22	171	387	1.8	34	208
	6/F		None	N2O + 1.5% Halothane	107	35	229	460	4.1	34	184
Kay et al,1986	6/F	2.5 mg/kg bolus	Diazepam 10 mg	N2O + 1.5% Halothane	109	42	329	708	2.4	56	262
	6/M	2.5 mg/kg bolus	Diazepam 10 mg	N2O + 1.5% Halothane	108	36	313	801	2.2	45	309

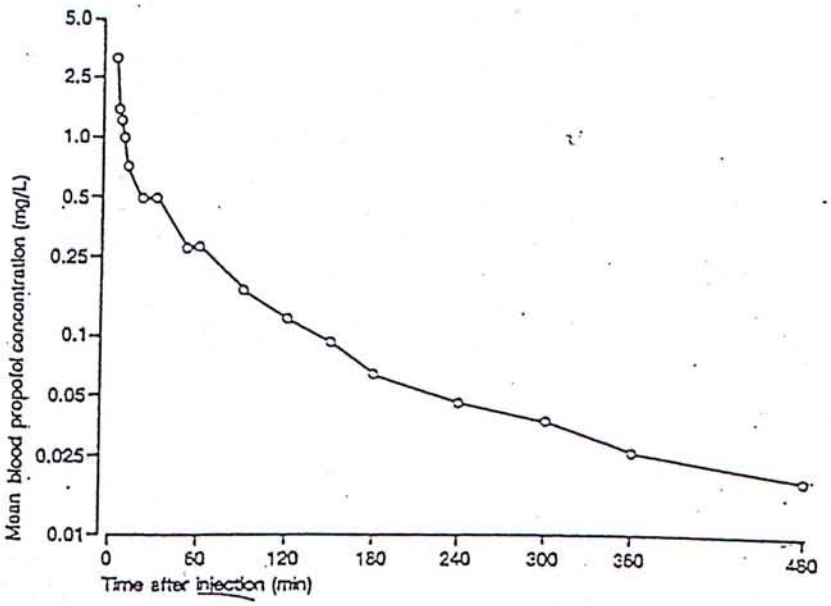

Fig 2-3: Mean blood propofol concentration in 6 female patients with normal hepatic and renal function following induction doses of propofol 2.5 mg/kg (after Cockshott 1985).

2.3: Effects of age, sex, and hepatic and renal disease on the pharmacokinetics of propofol

2.3.1: Effects of age

The reduction in clearance suggests that metabolism of propofol is reduced in the elderly, possibly as a result of the reduction in hepatic blood flow that occurs with age. Blood concentrations of propofol at 2 minutes after injection were significantly higher in the elderly patients (6.1 *vs* 4.2 mg/L; $p < 0.02$). This is partly a result of the decrease in V_c (The central volume of distribution) in these patients and probably explains why the elderly are more sensitive to propofol (Dundee *et at.* 1986). The pharmacokinetics of propofol were obtained for 12 elderly patients (65 to 80 years) who received single bolus injection of propofol 2 mg/kg and were compared with those obtained from a control group of 12 younger patients (18 to 35 years) who received doses of 2.5 mg/kg. In both groups papaveretum (10 to 20 mg intramuscularly) was given 1 hour before surgery and anaesthesia was maintained with up to 1 % halothane/67% nitrous oxide in oxygen. For both study groups the data were fitted to an open 3-compartment model. The terminal elimination half-life was similar for both the elderly (834 minutes) and younger patients (674 minutes), but propofol clearance was significantly reduced in the elderly 86 *vs* 107 L/h; $p < 0.05$). The initial volume of distribution (V_c) was significantly reduced in the elderly

(19.6 *vs* 26.3 L; $p < 0.05$) (Kirkpatrick *et al.* 1988).

2.3.2: Effects of Sex

Kay and associates (1986) compared the pharmacokinetics of propofol in male and female surgical patients and found no significant differences in clearance, volumes of distribution and distribution and elimination half-lives. The ratio of volume of distribution at steady-state (V_{ss}) to volume of distribution during elimination (V_d) was relatively low in these patients (0.58 or less), again probably as a result of retention of propofol in poorly perfused fat deposits, but it was significantly higher in men than in women ($p = 0.021$; Kay *et at.* 1986).

2.3.3: Effects of renal and hepatic disease

Mean distribution and elimination half-lives were measured in 4 patients with renal insufficiency who were undergoing abdominal surgery and were found to be slightly lower than in a similar group of 6 patients with normal renal function, but the differences were not statistically significant. In addition, total propofol clearance values were similar in the 2 groups (renal insufficiency, 111 L/h; normal renal function, 100 L/h) (Morcos & Payne 1985), and on the basis of these limited findings it would seem that renal disease does not seriously alter propofol pharmacokinetics.

The liver is the most important site of elimination,

and very high clearance values, of 70-140 L/hr have been reported (Sebel *et al.* 1989). The pharmacokinetics of an induction dose of propofol 2.5 mg/kg in 10 patients with uncomplicated liver cirrhosis were compared with a group of 10 patients with normal liver function. No significant pharmacokinetic differences were found between the groups, and the authors suggested that extrahepatic metabolism of propofol may compensate for reduced liver function (Servin *et al.* 1988). Servin and coworkers (1990) have compared the pharmacokinetics of propofol as an infusion in 10 control and 10 patients with cirrhosis. Total body clearance was not reduced significantly in cirrhotic (1.56 ± 0.48 L/min) compared with control (1.75 ± 0.32 L/min) patients. The volume of distribution at steady state was significantly greater in patients with cirrhosis than in control patients (202 ± 82 L *vs* 121 ± 49 L). However, this difference did not significantly change terminal elimination half-life. The pharmacokinetics of propofol given by infusion to maintain general anaesthesia were not affected markedly by moderate cirrhosis.

3: Pharmacodynamic

3.1: Anaesthetic concentrations

Studies designed to determine the minimum infusion rate of propofol needed to suppress the initial response to surgical incision provide data on blood concentration requirements for anaesthesia. Fifty-seven patients, premedicated with morphine 0.15 mg/kg, had anaesthesia induced with 2 mg/kg of propofol, supplemented by 67 % nitrous oxide in oxygen (Spelina *et al.* 1986). When a propofol infusion had been in progress for at least 30 minutes, at which time blood concentrations approximated to steady state conditions (Coates *et al.* 1987), the response to the initial surgical stimulus was noted. The mean whole blood propofol concentrations required to suppress movement in 50 % and 95 % of patient (ED_{50} and ED_{95}) were 1.67 μg/ml and 3.38 μg/ml, respectively. Higher concentrations (ED_{50} 2.5 μg/ml; ED_{95} 5.92 μg/ml) were required to suppress movement at incision following a benzodiazepine premedication (Turtle *et al.* 1987).

Following administration of single bolus doses or after stopping infusions of propofol, patients wake at a mean blood propofol concentration of approximately 1.0 μg/ml. Waking occurred about 10 minutes after a single bolus dose of 200 mg at a mean blood propofol concentration of 1.1 μg/ml (range 1.0 to 2.19 μg/ml) in the absence of

other drugs (Schüttler *et al*, 1985). Propofol was administered as a bolus dose, 2 mg/kg intravenous, followed by a variable-rate infusion, 0-20 mg/min, and intermittent supplemental boluses, 10-20 mg intravenous. Blood propofol concentrations at which 50 % of patients (EC50) were awake and oriented after surgery were 1.07 and 0.95 μg/ml (Shafer *et al*, 1988).

3.2: Recovery characteristics

The need to make more efficient use of hospital resources has led to an increasing number of minor surgical procedures being carried out on an out-patient basis. In such circumstances an important requirement is that recovery from anaesthesia should be rapid with minimal residual effect. The wealth of favourable data generated from clinical studies has encouraged widespread use of propofol in this field. A number of studies have compared methohexitone with propofol for induction and maintenance, or for induction only followed by inhalation anaesthesia. Immediate recovery times were either equivalent for the two drugs or were shorter after propofol, with the exception of one study where single bolus injections were used to facilitate dental extractions (Logan *et al*. 1987)

Although recovery from anaesthesia with either propofol or methohexitone was sufficient to allow patients

to be sent home on the same day as surgery was performed, psychomotor performance tests showed that functional impairment was less prolonged after propofol. Walking ability and correct balance also returned more quickly after propofol anaesthesia, whilst nausea and vomiting were more frequent in the patients who received methohexitone. Overall assessments of anaesthesia and recovery generally found propofol to be the superior drug. Results from take-home questionnaires which were used in one study indicated that patients generally felt better after propofol anaesthesia and that this difference persisted after discharge (Kay & Healy 1985). Isoflurane has been compared with propofol for the maintenance of anaesthesia following induction with propofol in outpatient surgical procedures. Again, although both provided adequate anaesthesia and rapid recovery, psychomotor performance improved more rapidly after maintenance with propofol (Milligan *et al.* 1987).

3.3: Effects on the cardiovascular system

Propofol causes a significant fall in arterial blood pressure and total peripheral resistance, with a slight fall in cardiac output. The heart rate may increase. McCollum and Dundee (1986) compared the effects of propofol 2-2.5 mg/kg, thiopental 4-5 mg/kg, methothexital 1.5 mg/kg,

and etomidate 0.3 mg/kg, given over 20 seconds to unpremedicated patients. Administration of 2.5 mg/kg propofol produced the greatest mean decrease in systolic blood pressure (-17 %) at 2 minutes, being significantly greater than that produced by 5 mg/kg thiopental. Grounds *et al.* (1985) reported that the 32 % fall in mean blood pressure observed 2 minutes after induction of anaesthesia with 2 mg/kg propofol resulted from a 13 % decrease in cardiac output and a 21 % fall in systemic vascular resistance, with minimal changes in heart rate. These changes were not significantly greater than those produced by thiopental 4 mg/kg.

Coates *et al.* (1987) used an induction dose of 2 mg/kg, followed by a continuous infusion of 3.24 mg/kg/h to supplement anaesthesia, with 67 % nitrous oxide in oxygen for body surface surgery and reported similar hemodynamic alterations. There were no further significant changes in systolic or diastolic blood pressure during the infusion, but cardiac output decreased to 65 % of the awake value. The introduction of positive pressure ventilation was associated with a significant increase in systemic vascular resistance compared with the value for spontaneously breathing patients, but no other hemodynamic indices showed any significant variation.

Twenty patients were divided randomly for administration of 2 mg/kg propofol (group Propofol, n = 10) or 0.9% saline solution (group Control, n = 10) during

cardiopulmonary bypass (CPB). The two groups were comparable with respect to sex, age, height, type of surgery (valvular or coronary), arterial hypertension and preoperative antihypertensive treatment. Mean arterial pressure (MAP), systemic vascular resistance (SVR) and SVR index were significantly lower in the propofol group than in the control group at 10, 15 and 20 min of study, suggesting that the hypotensive effect of a bolus injection of propofol is due, at least in part, to a direct decrease in the SVR (Pensado *et al*, 1993).

The effects on corrected QT interval (QTc), heart rate and arterial pressure were studied after induction with propofol 1.5, 2 or 2.5 mg/kg, thiopentone 5 mg/kg or methohexitone 2 mg/kg in 123 ASA class I or II children undergoing outpatient otolaryngological surgery. After injection of the intravenous anaesthetic, the QTc interval was significantly prolonged after propofol 2.5 mg/kg. Thirty seconds after suxamethonium 1.5 mg/kg, a significant prolongation of the QTc interval occurred in the thiopentone and propofol 1.5 and 2 mg/kg groups. After intubation, no further prolongation of the QTc interval occurred in any of the groups. Heart rate increased significantly after the barbiturates but not after propofol. Systolic arterial pressure decreased significantly after propofol 1.5 and 2.5 mg/kg. In all groups a cardiovascular intubation response occurred. Bradycardia and junctional rhythm occurred in 4% of the

children in both barbiturate groups and in 19-29% in the propofol groups. It is concluded that propofol causes prolongation of the QT interval and results in a higher incidence of bradycardia and junctional rhythm than the barbiturates (Saarnivaara *et al*, 1993).

3.4: Effects on the respiratory system

Propofol is a respiratory depressant. A bolus dose of propofol may cause a decrease in tidal volume, leading to apnoea in many patients. This has been a consistent observation in clinical studies where propofol has been used to induce anaesthesia, and although the duration of apnoea is usually short (60 seconds or less) it may persist for up to 3 minutes (Goodman *et al*. 1987). Similarly, the incidence of apnoea varies considerably, occurring in between 50 and 84% of patients and this may be dependent upon a variety of factors, such as premedication, speed of administration, dose, hyperventilation and hypercapnia.

Detailed respiratory measurements in 16 unpremedicated surgical patients who received an induction dose of propofol 2.5 mg/kg revealed that the apnoea was preceded by a rapid reduction in tidal volume (usually accompanied by tachypnoea) which was maximal about 30 seconds after injection and then progressed into full apnoea. Breathing then resumed spontaneously with small tidal volumes which

increased over a period of about 1 minute to a steady rate (Goodman *et al.* 1987).

Both Vt and Vi were decreased during infusion at 6 mg/kg/h and showed a further reduction when the infusion rate was doubled, whilst the respiratory frequency increased to a maximum during the postinduction period then decreased slightly during the constant infusion. The inspiratory duty cycle (T_i/T_{tot} - the ratio of inspiratory time to total ventilatory cycle time) was reduced during the lower rate infusion and further decreased when the rate was increased. In addition, analysis of the ventilatory response to rebreathed carbon dioxide in 8 of the patients indicated that an infusion of propofol 6 mg/kg/h reduced this response (determined as the gradient of the carbon dioxide rebreathing curve) to an average of 58 %, within 95% confidence limits of 32 and 84 % (Goodman *et al.* 1987).

3.5: Effects on cerebral blood flow and intracranial pressure

In patients without intracranial pathology, propofol decreases cerebral blood flow (CBF) by 26% (Vandesteene *et al.* 1988) to 51% (Stephan *et al.* 1987), and increases cerebrovascular resistance (CVR) by 51% to 55%. Propofol was found to decrease cerebral metabolic requirement for oxygen to a significant degree (36%) in one study (Stephan

et *al*. 1987), but insignificantly (18%) in another study (Vandesteene *et al*. 1988), which combined a propofol infusion with nitrous oxide (65%), oxygen, and enflurane (0.5%). The reactivity of the cerebral vessels to changes in Pco_2 seems to be maintained during anaesthesia with propofol. The effects of propofol in patients with elevated intracranial pressure (ICP) have been studied with propofol (2 mg/kg) administered to six comatose patients with ICP greater than 25 mmHg. In this group of patient, ICP decreased significantly. However, the cerebral perfusion pressure (CCP) also decreased in four of six patients below the minimum safe CCP of 50 mmHg due to a decrease in arterial pressure (Herregods *et al*. 1988).

3.6: Other effects

3.6.1: Effects on liver function

Propofol has minimal adverse effects on liver function, as evidenced by the absence of change in liver function tests such as aspartate transaminase (AST), alanine transaminase (ALT), or alkaline phosphatase, up to 15 days after a general anaesthetic with propofol and nitrous oxide (Robinson *et al*. 1985). The pharmacokinetics of propofol given by infusion were not affected markedly by moderate cirrhosis (Servin *et al*. 1990).

3.6.2: Effects on renal function

Propofol has not been reported to have adverse effects on renal function. In uraemic patients undergoing renal transplantation (Kirvela *et al.* 1991), the pharmacokinetics of propofol, following a single bolus injection of propofol (2.0 mg/kg), were similar to those in patients with normal kidney function.

3.6.3: Effects on coagulation

The emulsion in which propofol was reformulated resembles Intralipid, which has been associated with alterations of blood coagulation (Burnham *et al.* 1982). With one possible exception (Newman *et al.* 1987), studies have failed to show that propofol changes the coagulation profile as measured by thrombin time, prothrombin time, partial thromboplastin time, fibrinogen titre, fibrin degradation products, and platelet count (Kay *et al.* 1985). In addition, propofol was found to have no apparent effects on platelet function (Mayne *et al.* 1988).

3.6.4: Effects on adrenal steroidgenesis

In man, when used as a single induction dose of 2.5 mg/kg, propofol did not block cortisol and aldosterone secretion in response to surgical stress or ACTH (Fragen *et al.* 1987). In contrast, using the same dose of propofol for induction, followed by a continuous infusion (4.4 mg/kg/h), Kay and colleagues (1985) found a transient decrease in

plasma cortisol concentration 30 min after induction of anaesthesia (Kay *et al.* 1985), but cortisol concentration was not significantly different from baseline. Propofol was found to have no effect on plasma cortisol concentrations, and the response to the Synacthen test was normal, with a significant increase in cortisol concentration 30 and 120 min after Synacthen administration.

3.7: Side effects

3.7.1: Pain on injection

The most frequent side effect associated with the use of propofol has been pain on injection. Collation of data from a total of 1,465 patients found that injection of propofol into a vein in the dorsum of the hand (n = 428) was painful in 28.5% of cases. However, if the injection was into the large veins of the forearm or antecubital fossa (n = 821) this figure fell to 6% (Stark *et al.* 1985). A similar incidence was reported in a comparative study of propofol as an induction agent in unpremedicated patients. The incidence of pain on injection in the antecubital fossa and dorsum of the hand, respectively, were 8 and 31% for propofol (n = 100), 4 and 5% for thiopentone (n = 100), 14 and 29% for etomidate (n = 50) and 18 and 41% for methohexitone (n = 50)(McCollum & Dundee 1986).

3.7.2: Excitatory & respiratory

Excitatory and respiratory effects that occur during induction are generally considered as side effects of the anaesthetic, although their incidence will to some extent be dependent upon other drugs that are used and the skill of the anaesthetist. In the studies reviewed by Stark *et al.* (1985) excitatory effects (movement, twitching, hiccup, tremor etc.) were seen in 14% of 1459 inductions with propofol. Apnoeic episodes are more frequently seen; McCollum and Dundee (1986) reported that apnoea of more than 30 seconds duration occurred in 44% and 24% of unpremedicated patients who received propofol 2.5 and 2 mg/kg, respectively (n = 50 for each dose) In comparison, the figures for methohexitone 1.5 mg/kg, etomidate 0.3 mg/kg and thiopentone 5 mg/kg were 20, 0 and 38%, respectively. In some instances the duration of apnoea with propofol can exceed 60 seconds (Goodman *et al.* 1987); this will depend to some extent on the induction procedure (bolus dose or titrated increments), and it is likely to be exacerbated by the concomitant use of opioids.

3.7.3: Nausea and vomiting

A particular feature of propofol anaesthesia is the low occurrence of postoperative nausea and vomiting, with an overall incidence of about 2 to 3%. In the comparative studies included in this analysis 13% of patients who received thiopentone (n = 79) and 10% of patients who

received methohexitone (n = 86) either vomited or became nauseous (Stark *et al.* 1985).

3.7.4: Bradycardia

Isolated cases of bradycardia have been seen during propofol anaesthesia. These were often associated with surgical procedures that produce vagal stimulation (Henriksson *et al.* 1987) and were easily controlled by administration of atropine, although persistent bradycardia which was resistant to both atropine and isoprenaline (isoproterenol), and where heart rate recovered slowly only after propofol infusion was stopped has been reported in 1 patient (Thomson & Yate 1987).

3.7.5: Anaphylaxis

Fourteen patients who had a life-threatening reaction within a few minutes after receiving propofol (Diprivan) were investigated for anaphylaxis 4-6 weeks after the incident by Laxenaire *et al.* (1992). Three kinds of immunologic tests were carried out: skin tests (prick tests and intradermal tests with the drugs used and Intralipid, the solvent for propofol), a leukocyte histamine release test, and a radioimmunoassay (RIA) of immunoglobulin E (IgE) against propofol and muscle relaxants, when they had been given with propofol. It had been previously shown that these were always negative in patients anaesthetized with propofol without any complications. Thirteen of the 14

patients had at least one positive test supporting hypersensitivity to propofol; 2 patients had three tests positive; 4 had two tests positive; and 7 had one test positive. The skin tests with Intralipid were negative in 4 patients whose tests with propofol were positive. Two patients who had been given muscle relaxants at the same time as the propofol had positive IgE-RIA to both drugs. In one patient, results of all the tests remained negative, and the mechanism involved in the reaction remained unidentified. It is note-worthy that 9 patients of 14 had allergic histories that were known before the anaesthetic (atopy; allergy to antibiotics, muscle relaxants, lidocaine, colloids) and that none of the patients had ever received propofol or Intralipid before. It is possible that the IgE that linked abnormally with the propofol had specific binding sites for the phenyl nucleus and the isopropyl groups, which are present in propofol and many other drugs.

4: Clinical use

Propofol has proven to be a reliable anaesthetic that can be used for both induction and maintenance of anaesthesia in most common surgical procedures, either in 'standard' anaesthetic practice or as part of total intravenous anaesthesia. Comparative studies have show that

it is at least as effective as other intravenous anaesthetics in most respects, with both potential advantages and disadvantages in individual situations. In outpatient surgery, rapid return to normal psychomotor function is clearly important. Propofol also offers a particularly low incidence of postoperative nausea and vomiting, which is desirable in any setting, but again may be especially beneficial in outpatient surgery. The incidence of excitatory effects during surgery under propofol anaesthesia is also low and propofol is superior to methohexitone in this regard. Propofol infusions, as not depress adrenal responsiveness to ACTH during short term administration, as occurs with etomidate.

Disadvantages of propofol include pain on injection, a relatively high incidence of apnoea, and blood pressure reductions that may occasionally be marked. However, in studies to date the magnitude of these effects was such that their management during anaesthesia was straightforward in most patients.

Propofol has been used for three main clinical applications;

a) Intravenous bolus for induction of anaesthesia.
b) Intravenous infusion or repeated bolus for maintenance of anaesthesia.
c) Intravenous infusion or repeated bolus for sedation.

4.1: Anaesthesia induction

The ideal intravenous induction agent possesses characteristics which include rapidity of onset and recovery, reliability of action (smooth onset without excitatory effects or respiratory distress), water solubility, lack of allergic responses and tissue toxicity, and lack of hemodynamic effects. Propofol possesses a high degree of lipophilicity and a short half-life which make it suitable for intravenous induction with rapid access to the brain and subsequent rapid recovery due to redistribution and elimination. Induction doses ranged from 2 to 3 mg/kg. In those over 60 years old, 1.5 mg/kg is adequate (Dundee *et al.* 1986). The induction dose is also reduced by premedication with benzodiazepines or opiates (Thomas *et al.* 1988).

Propofol has been compared with other intravenous and inhalation anaesthetics for induction of anaesthesia in a range of general surgical procedures. Comparisons with methohexitone for both induction and maintenance (in combination with nitrous oxide) always found propofol to be at least as effective as methohexitone, irrespective of premedication and the use of additional analgesics. Recovery from propofol anaesthesia was often significantly faster. Propofol seems to produce a higher incidence of apnoea on induction than methohexitone, but there is less movement during the induction period with propofol. The

latter was particularly apparent when no premedication or other drugs were given (analgesia being provided by regional blockade), when the incidence of movement was 75% with methohexitone but only 20% with propofol (Mackenzie & Grant 1985).

Propofol has also been compared with thiopentone for induction and again propofol was associated with significantly shorter recovery times and was found to be superior by anaesthetists' overall assessments (Henriksson *et al.* 1987) in short procedures.

4.2: Anaesthesia maintenance

Propofol is the only currently available intravenous anaesthetic that is suited for maintenance of anaesthesia. This is due to its favourable pharmacokinetic profile which avoids the accumulation seen with prolonged thiopental administration, the preservation of adrenal steroidogenesis (Kay *et al.* 1985)(in contrast with etomidate infusions), the ability to provide easily controllable depth of anaesthesia, and relative freedom from excitatory effects, which occur with alternative agents such as methohexital (Kay 1986).

Whether maintenance of anaesthesia was by intermittent bolus injections or continuous infusions of propofol, analgesia provided with nitrous oxide, alone or in

combination with opioids, recovery has been rapid in all studies (mean recovery time of 15 minutes or less) (Mark *et al.* 1988), despite a varying duration of anaesthesia from 6 minutes up to 3 hours.

Use of propofol for both induction and maintenance of anaesthesia was also reported to be a suitable alternative to induction with thiopentone and maintenance with inhalation anaesthetics halothane, isoflurane and enflurane and recovery was often quicker after propofol anaesthesia. Thus, propofol compares well with other intravenous and inhalation anaesthetics in a wide range of general surgical procedures, and results in more rapid recovery in most situations. Several authors have commented additionally on the good 'quality of recovery' after propofol, with regard to clear headedness and alertness (Henriksson *et al.* 1987; Mackenzie & Grant 1985).

The suitability of propofol for infusion, together with important advances in infusion technology, have resulted in an upsurge of interest in total intravenous anaesthesia. The technique has a number of important practical advantages over inhalation methods, including the provision of good surgical access in oral and airway procedures, the ability to avoid nitrous oxide, and compatibility with jet ventilation and airway laser surgery techniques. Total intravenous anaesthesia with propofol provides a versatile and effective anaesthetic technique which avoids the pollution of the working environment

caused by the use of inhalational agents.

Coates and coworkers described dose requirements effective dose 50 (ED_{50}) of 3.24 mg/kg/h and effective dose 95 (ED_{95}) of 5.46 mg/kg/h. The average rate of infusion varied and was reported as high as 11.5 mg/kg/h (Coates *et al.* 1985).

Infusions of propofol and an opioid (without nitrous oxide) have been combined as general anaesthesia. Combined infusions were first reported in 1985 by DeGrood *et al.* using fentanyl and propofol with good success at controlling anaesthetic depth and hemodynamic changes. Because of their similar pharmacodynamic characteristics (rapid onset and short duration of action), it seems that propofol and alfentanil would be ideal and complementary agents for total intravenous anaesthesia. When these two agents were administered together for orthopaedic or ear, nose and throat procedures, anaesthesia proceeded smoothly with predictable changes in hemodynamics after induction and intubation. Emergence from anaesthesia was rapid (time from end of infusion until patient being oriented: 12.8-18.9 minutes). In the recovery room patients were described as fully alert and clear headed (Steegers *et al.* 1988).

If propofol is to be used by continuous intravenous infusion, then it is likely that sophisticated microprocessor-controlled infusion devices will allow stable plasma concentrations of the drug to be achieved rapidly. An initial assessment of such an infusion device,

which was aimed at a constant blood level of 2.5 μg/ml, demonstrated that stable concentrations of the drug could be achieved. Taking the 20 patients studied as a group, induction of anaesthesia was not associated with hypotension. The time taken by the microprocessor to achieve stable concentrations was not reported (Schuttler *et al.* 1988). A similar study was carried out using a different microprocessor system aiming for a target concentration of 3 μg/ml (Roberts *et al.* 1988). This concentration was reached within 10 minutes. The blood pressure and heart rate decreased over the first 15 minutes of the study. It is also possible to achieve a stable plasma concentration rapidly using a manually controlled infusion scheme. This involved making four rate adjustments in the first 20 minutes, but achieved stable concentrations within 2 minutes of start of infusion. Again, these authors suggested that this technique provided better hemodynamic stability during induction than a loading dose of 2 mg/kg. Computer-controlled infusion schemes will result in a stable mean concentration of drug in a group of patients. However, the standard deviation may be as large as 30% of the target level (Tackley *et al.* 1987; Roberts *et al.* 1988).

4.3: Use in sedation

Sedation of critically ill patients in the intensive care setting traditionally involves the use of benzodiazepines and opioids, which may cause prolonged recovery, respiratory depression, and gastrointestinal stasis. There have been a number of reports of the successful use of propofol by continuous infusion for sedation in intensive care unit patients with a variety of illnesses, including cardiac surgery, head injury, acute respiratory failure, septic shock, and acute tubular necrosis (Robert & Deegan 1992). The duration of sedation was 8-189 hours and the mean dosages used varied from 0.79 to 2.88 mg/kg/hr, reflecting the different ages and conditions of the patients in the studies. In each study, adequate sedation was achieved without difficulty. Supplemental opioid boluses were required in each case but significantly less than with midazolam sedation. Recovery was also very satisfactory (Robert & Deegan 1992).

Administration of propofol by continuous infusion or intermittent bolus has been found to be suitable for sedation during regional anaesthesia or during day case endoscopies, comparing favourably with midazolam and methohexital (Fanard *et al.* 1988, Dubois *et al.* 1988).

CHAPTER THREE

ANALYTICAL TECHNIQUE:

PROPOFOL CONTENT ANALYSIS

1: Introduction high-pressure liquid chromatography

High pressure liquid chromatography (HPLC) has become the fastest growing analytical technique. The practice of liquid chromatography developed from open column, gravity fed systems utilizing effluent collection, to using various analytical techniques, such as evaporation to dryness and subsequent dilution and/or reactions followed by ultraviolet-visible and infrared spectroscopy, to a totally instrumental methodology. Decreases in column packing diameters produced proportional increases in solvent flow resistance, and as a result, constant flow and constant pressure liquid pumping systems were developed and introduced. Sample introduction by simply adding the sample to the head of the column was no longer practical, and liquid syringe and valve injectors were added for sample introduction. The efficiency of HPLC allowed very small

samples to be used, and the classical methods of collecting effluents for further analysis were replaced by using low dead volume detectors coupled with strip chart recorders.

The development of small diameter packing materials and columns allowed the chromatographer to perform separations faster and with greater resolution than had previously been attainable. HPLC has become the most popular chromatographic method, because HPLC is not limited to sample volatility and thermal stability as is gas chromatography. Nearly all classes of organic compounds can be separated by this efficient and faster analytical technique.

The previously reported method for propofol using high-pressure liquid chromatography and ultraviolet detection of the indophenol derivative (Adam *et al.* 1981) was not considered sufficiently sensitive (the limit of quantification was 15 ng/ml) for the monitoring of all clinical studies. The combination of high-pressure liquid chromatography and fluorescence detection has improved the sensitivity of propofol measurements. The method described in this chapter is more rapid and simpler to use than the indophenol procedure and has improved the sensitivity of analysis, with the lower limit of quantification being 2 ng/ml using a blood sample volume of 0.5 ml.

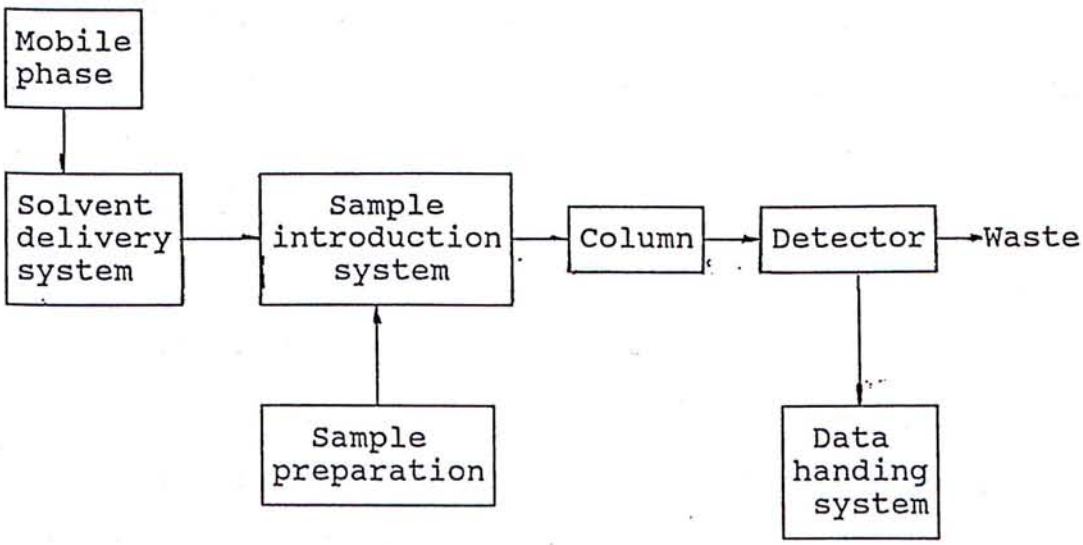

Fig 3-1: HPLC system diagram

2: Methods of propofol content analysis

Refer Appendix for source & manufacturer of all reagents and equipment.

This method was based on that published by Chan & So 1990 with slight changes in the mobile phase composition.

2.1: Reagents and solutions.

2.1.1: Sodium dihydrogen phosphate 0.1M M=137.99 g/mol

Take 1.38 g $NaH_2PO_4.H_2O$ dissolved in Mill-Q water Adjusted to pH 4.66-4.7 by PH-Meter with dilute Sodium hydroxide solution or Hydrochloric Acid and then diluted to 100 ml with Mill-Q water.

2.1.2: Cyclohexane $CH_2.(CH_2)4.CH_2$=84.16

2.1.3: Tetramethylammonium Hydroxide Solution.

Take 0.375 g Tetramethylammonium Hydroxide solid dissolve in 1.5 ml absolute Methanol and then dilute with 18.5 ml 2-Propanol (IPA).

2.1.4: Acetonitrile CH_3CN

2.1.5: Acetic acid CH_3COOH

2.2: Standard solutions.

2.2.1: Propofol standards.

Weigh a drop of 99% pure propofol stock solutions dissolved in 100 ml absolute methanol. Using this stock solution according $C_1V_1=C_2V_2$, prepare 3 standard solution containing 100, 10, 1 mcg/ml, stored at 4°C, but prepared fresh each two months.

2.2.2: Internal standard.

0.0012 g Thymol Crystals dissolved in 100 ml absolute methanol. Take a small aliquot and then dilute to 10 times with absolute methanol to produce 12 ng/ml. Stored at 4°C, also prepared fresh each two months.

2.2.3: Control standard.

Take 40 ml of whole blood and divide into 2 tubes. In one tube, add 20 µl of 100 mcg/ml propofol standard solution to prepare 100 ng/ml control. In the other tube, add 100 µl of 100 mcg/ml propofol standard solution to prepare 500 ng/ml control. Mix for one hour at room temperature and transfer 0.7 ml aliquots of the prepared control levels into 1.5 ml eppendorf tubes and store at 4°C for later use.

2.3: Mobile phase

Take 660 ml Acetonitrile, add 3.4 ml Acetic acid and dilute to 1 litre with Mill-Q water. The mobile phase is mixed for one hour at room temperature using a magnetic bar on stir plate mixer, filtered with filter paper of type GV with 0.22 μm pore size and degassed in an ultrasonic cleaner.

2.4: High-pressure liquid chromatography

The high-pressure liquid chromatography apparatus consisted of a metering pump solvent-delivery system set to deliver a solvent (66% Acetonitrile as described **2.3:** Mobile phase) at a flow-rate of 1.7 ml/min and 700 PSI operating pressure. A metering pump was used in conjunction with an injection system and a column Nova-pak C18 ODS in a Waters RCM 8×10 radial compression module. The fluorescence spectrophotometer was used at excitation wavelength of 276 nm & emission wavelength of 310 nm, and both monochromator slit widths were 10 nm. The fluorescence spectrophotometer was attached to an integrator and the signals were recorded using a hot-pen chart recorder.

2.5: Quantification

Calibration standards were prepared in expired whole blood by addition of known amounts of propofol (50, 100, 250, 500, 1000, 3000 ng/ml) and a constant amount of thymol (50 μl, 12 ng/ml) corresponding to the median concentration of the calibration series under examination. The standards were treated in an identical manner to the unknown samples. The ratio of the peak heights derived from propofol and thymol in the standards were used to construct calibration curves from which unknowns were quantified. Each concentration was done in triplicate.

A calibration graph was prepared by the addition of known quantities of propofol to aliquot of control blood and extracted according to the below **2.6:** Procedure. The peak-height ratio of propofol to thymol was plotted against the concentration of propofol and the concentration of propofol in the samples was calculated using the regression parameters obtained from the calibration graph.

Table 3-1:

Calibration of Propofol standard in Whole blood

Propofol ng/ml	Peak height of Propofol	Peak height of Internal standard	Ratio	Mean	S.D.	C.V. (%)
50A	201652	2077037	0.0971			
50B	257874	2534218	0.1018	0.0990	0.0018	1.83
50C	233445	2375293	0.0983			
100A	490064	2588574	0.1893			
100B	418834	2060215	0.2033	0.1945	0.0059	3.03
100C	505763	2651274	0.1908			
250A	875590	1775794	0.4931			
250B	1168430	2282474	0.5119	0.4997	0.0081	1.63
250C	1100427	2227067	0.4941			
500A	1899784	2171686	0.8748			
500B	2259874	2406278	0.9392	0.9114	0.0244	2.68
500C	2059410	2237619	0.9204			
1000A	3029338	1516818	1.9972			
1000B	3888829	2061912	1.8860	1.8744	0.0896	4.78
1000C	3846022	2210350	1.7400			
2000A	3418218	900556	3.7957			
2000B	2147245	600193	3.5776	3.7272	0.0997	2.68
2000C	2906402	763161	3.8084			
3000A	5426330	963884	5.6297			
3000B	3680522	601426	6.1197	5.7548	0.2433	4.23
3000C	3742930	678689	5.5149			

Regression Output:			
Constant	-0.01079		y = mx + b
Std Err of Y Est	0.12725		r = 0.9962
R Squared	0.99625		m = 0.001904
No. of Observations	21		b = -0.01079
Degrees of Freedom	19		f = 525
X Coefficient(s)	0.001904		
Std Err of Coef.	0.000027		

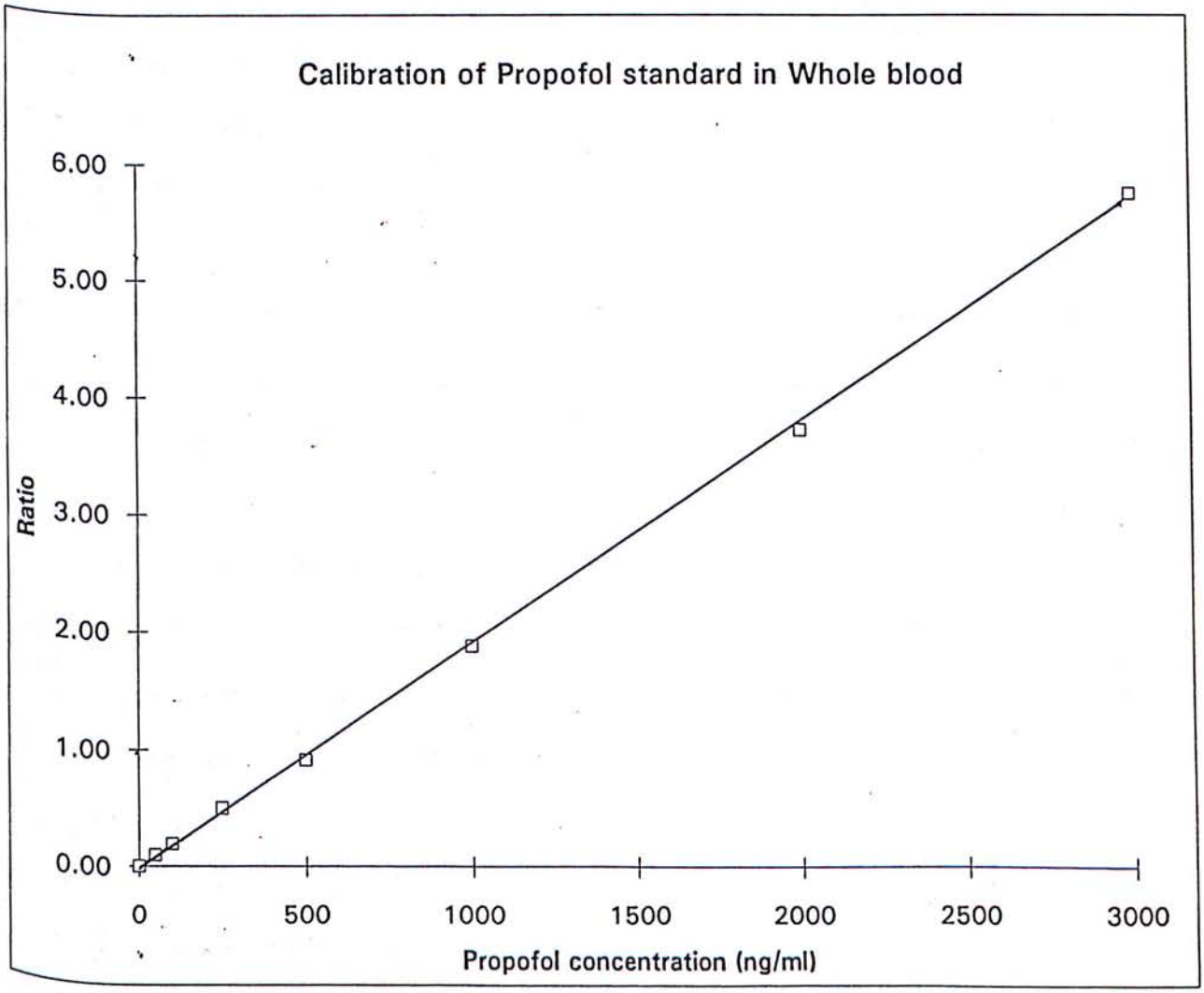

Fig 3-2: Calibration of Propofol standard in Whole blood

2.6: Procedure

Samples were whole blood in heparinized tube stored at 4°C, until analysis. 0.5 ml heparinized whole blood was transferred into a 15 ml silanized and screw-cap glass tube. To this was added 50 μl internal standard (as described **2.2.2:** Internal standard) and 1 ml (0.1 M) Sodium dihydrogen phosphate (PH 4.6) (as described **2.1.1:** Sodium dihydrogen phosphate) and 5 ml cyclohexane (as described **2.1.2:** Cyclohexane). The tube was screw-capped and the mixture was tumbled (33 rpm, 15 min) in a rotary tumbler. It was then centrifuged for 5 minutes at 3000 rpm, and at 20°C. After centrifugation, the upper organic extract was transferred into a tapered, silanized evaporating tube containing 50 μl of Tetramethylammonium Hydroxide solution (as described **2.1.3:** Tetramethylammonium Hydroxide Solution), and the cyclohexane extract was evaporated to dryness under a stream of nitrogen gas at 37°C in a water bath. The dried residue was dissolved in 80 μl acetonitrile (as described **2.1.4:** Acetonitrile) and stored in an ice bath. An aliquot (10-50 μl) of the solute was submitted to high-pressure liquid chromatography analysis. Typical chromatograms of extracts containing propofol are shown in **Fig 3-3:** Chromatogram of extracts containing propofol & internal standard.

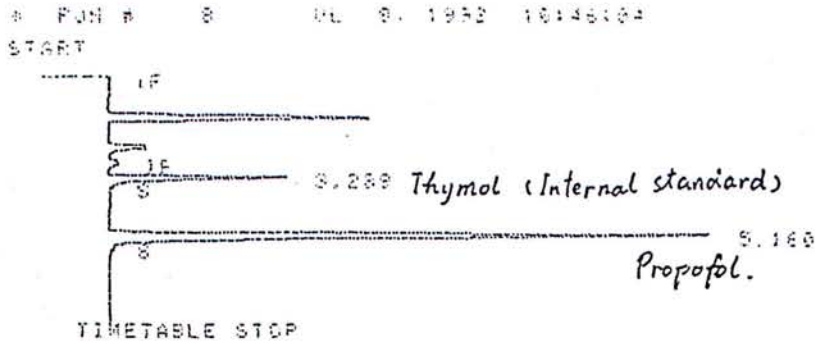


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Fig 3-3: Chromatogram of extracts containing propofol & internal standard.

$$\text{Ratio} = \frac{\text{Concentration of propofol}}{\text{Concentration of Internal standard}}$$

$$= \frac{\text{Peak height of propofol}}{\text{Peak height of Internal standard}}$$

$$= \frac{4402941}{1304343}$$

$$= 3.3756$$

Concentration of propofol = Ratio x f

= 3.3756 x 525

= 1772.19 ng/ml

2.7: Throughput:

2.7.1: First working day.

a) Prepared mobile phase: Diluted, mixed, filtered and degassed (as described **2.3:** Mobile phase).

b) Cleaning the high-pressure liquid chromatography system, using methanol with a flow-rate of 0.1 ml/min overnight.

c) Prepared the propofol standard, the internal standard and the control standard (as described **2.2:** Standard solution).

2.7.2: Second working day.

a) Calibration standards added a known amount of propofol (50, 100, 250, 500, 1000, 3000 ng/ml) and a known amount of thymol (50 μl 12 ng/ml) to 0.5 ml of expired whole blood. Each concentration was done in triplicate. Total of 18 standards. (as described **2.6:** Procedure)

b) All the prepared calibration standard were then injected to HPLC system. The total run time per trial was 8 minutes. Some calibration standards were reinjected if necessary. The time required to finish the HPLC run for all the standards is about 4 hours.

2.7.3: Third working day.

The peak-height ratio of propofol to thymol was calculated and a calibration graph was obtained from the above calculated result (as described **2.5:** Quantification).

The regression parameters was calculated at the same time.

2.7.4: Fourth working day.

a) Concentration of unknown samples were determined by preparing a set of calibration standards containing 3 different concentrations of propofol along with 2 control standards and 15 unknown samples from patients. (as described in **2.6:** Procedure).

b) All the prepared calibration standards, control standards and unknown samples were injected into the HPLC system with a run time of 8 minutes per injection.

2.7.5: Fifth working day.

The concentration of propofol in the unknown samples was calculated using the regression parameters obtained from the calibration graph. A total of 20 samples (15 test samples add 3 standards and 2 control standards) were tested.

The remaining unknown samples were processed in a similar manner.

3: Results of propofol content analysis

3.1: Calibration standard of propofol (Linearity)

The propofol standards, control standards and internal standards were prepared fresh every two months.

In each of my experiments, I included 3 propofol standards (50, 250, 1000 ng/ml propofol) and 2 control standard (100, 500 ng/ml propofol) with the test samples and the results were consistent. A linear relationship was obtained between the ratio of the peak height of propofol to that of thymol and the amount of propofol added to blood over the range 2-3000 ng/ml. Specimens with propofol concentrations above 3000 ng/ml was diluted to decrease the propofol concentration to within the calibrated range of the assay. No change in the slope was observed with different batches of blood used. These results indicate that the method permits the determination of propofol in blood over wide ranges of concentration. The calibration graphs were linear over the range 2-3000 ng/ml with coefficients of variation ranging from 0.3774 to 7.8617%. The between-batch coefficient of variance was 9.7850% at 50 ng/ml, 8.5220% at 100 ng/ml, 8.1776% at 250 ng/ml, 5.1353% at 500 ng/ml, 4.2039% at 1000 ng/ml, and 7.6469% at 3000 ng/ml, while the limit of detection was approximately 2 ng/ml.

Table 3-2:

Calibration of Propofol standard solution in Whole blood

The between-batch coefficient of variance

Propofol Conc. (ng/ml)	C-1A	C-2A	C-3A	C-4A	C-5A	Mean	S.D.	C.V. (%)
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
50	0.1100	0.1067	0.0928	0.0990	0.1234	0.1064	0.0104	9.7850
100	0.2333	0.2167	0.1837	0.1945	0.2186	0.2094	0.0178	8.5220
250	0.5200	0.4267	0.4562	0.4997	0.5328	0.4871	0.0398	8.1776
500	1.0367	0.9567	0.8965	0.9114	0.9517	0.9506	0.0488	5.1353
1000	1.8933	2.0833	1.8751	1.8744	1.8853	1.9223	0.0808	4.2039
3000	6.7833	6.2633	5.4636	5.7548	5.8269	6.0184	0.4602	7.6469

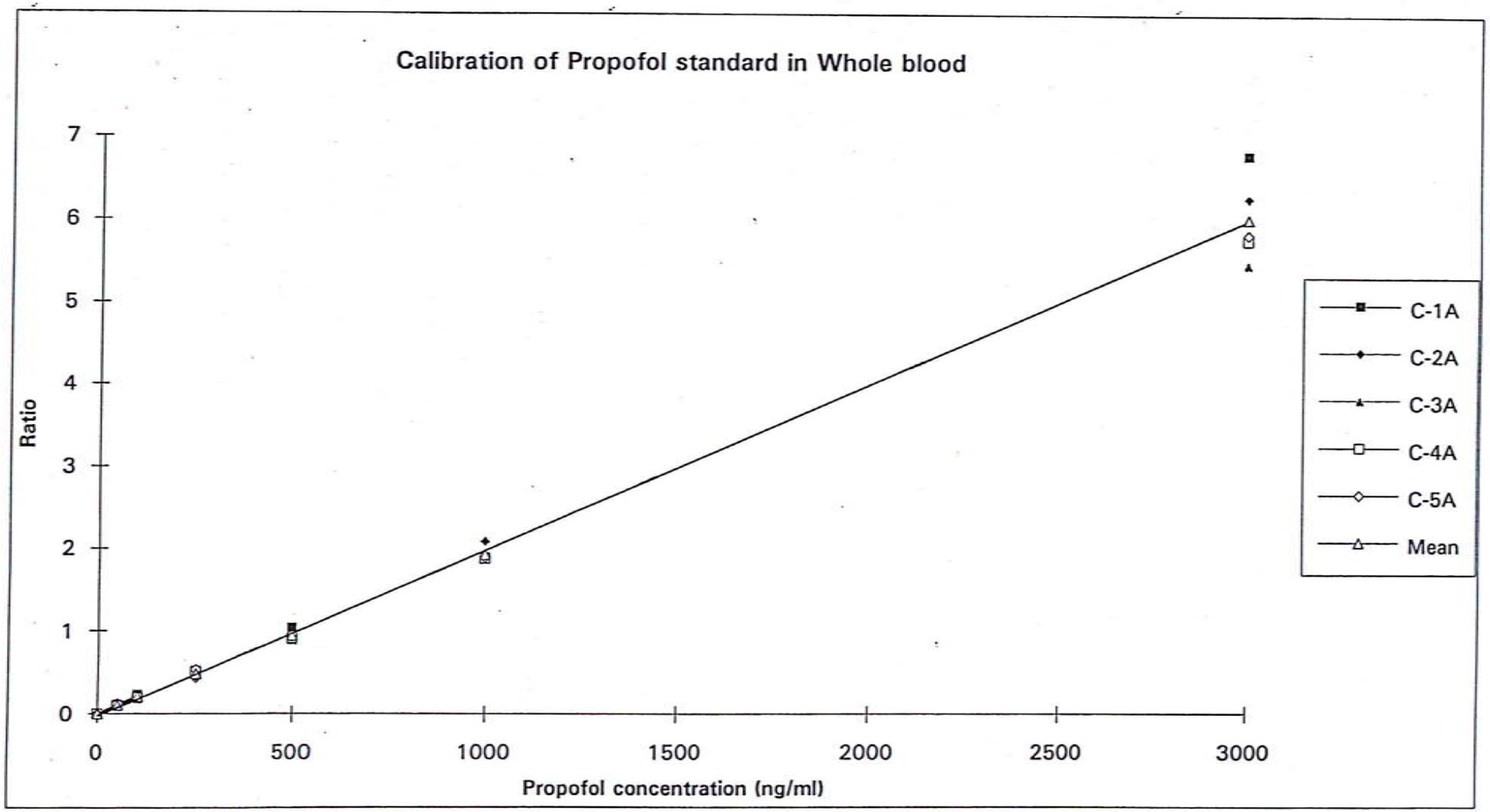


Fig 3-4: Calibration of Propofol standard in Whole blood (five-batches)

3.2: Control standard of propofol

3.2.1: Reproducibility

Pooled control whole blood is spiked with known concentration of propofol (100 ng/ml and 500 ng/ml) stored at 4°C. These pooled controls were assayed for propofol content on Day 0, 1, 3, 5, 7, 14, 21, 30, 60, 90. (1 trial each day).

3.2.1.1: Intraassay coefficient of variation (one analysis day)

The intraassay coefficient of variation (n = 5) of propofol in whole blood, which is done on one analysis day, was 6.24% at 100 ng/ml and 6.10% at 500 ng/ml (described in **Table 3-3:** Intraassay coefficient of variance and recovery for propofol in whole blood).

3.2.1.2: Interassay coefficient of variation (10 analysis days)

The interassay coefficient of variation (n = 10) for propofol in whole blood, which is done on ten analysis days, was 3.18% at 100 ng/ml and 1.81% at 500 ng/ml (described in **Table 3-4:** Interassay coefficient of variance for propofol in whole blood).

Table 3-3:

Intraassay coefficient of variance and Recovery for Propofol in Whole blood

Day	Sample	Concentration (ng/ml)		Recovery	Concentration (ng/ml)		Recovery
		Expect	Tested	(%)	Expect	Tested	(%)
0	Sample-1	100	100	100	500	521	104
	Sample-2	100	100	100	500	459	92
	Sample-3	100	96	96	500	468	94
	Sample-4	100	110	110	500	483	97
	Sample-5	100	115	115	500	545	109
Mean (n = 5)			104	104		495	99
S.D.			6.50	6.50		30.20	6.04
C.V. (%)			6.24	6.24		6.10	6.10

Table 3-4:

Interassay coefficient of variance for Propofol in Whole blood

Day	Control Low Concentration (ng/ml)	Control High Concentration (ng/ml)
0	100	483
1	102	505
3	101	487
5	100	489
7	100	488
14	96	490
30	90	457
Mean (n = 7)	98	485
S.D.	3.12	8.81
C.V. (%)	3.18	1.81

3.2.2: Recovery

The extraction efficiency can be estimated by comparing the results of experimental and the theoretical amount of Propofol in whole blood. The theoretical value is based on the knowledge of the amount of Propofol spiked into the specimens. The experimental value is based on the amount obtained by comparing the peak height ratio of the spiked specimens against those from the calibration curve.

The extraction recovery results obtained (n = 5) for propofol relative to the internal standard (Thymol) in whole blood was 104% at 100 ng/ml and 99% at 500 ng/ml (described in **Table 3-3:** Intraassay coefficient of variance and recovery for propofol in whole blood).

3.2.3: Stability

Pooled whole blood is divided into two tubes. Known volumes of standard Propofol is spiked into these tubes to prepare pooled controls containing 100 ng/ml and 500 ng/ml of Propofol respectively. Aliquots of 0.7 ml were transferred into 1.5 ml eppendorf tubes and stored at 4°C for subsequent use on separate analysis days to check for stability during storage. The stability results obtained (n = 10) for propofol control in whole blood analysis. (described in **Fig. 3-5:** Stability of propofol control in whole blood)

Table 3-5:

Stability of Propofol Control standard in Whole blood

Day	Control Low Concentration (ng/ml)	Control High Concentration (ng/ml)
0	100	483
1	102	505
3	101	487
5	100	489
7	100	488
14	96	490
21	88	471
30	90	457
60	85	473
90	86	453
Mean (n = 10)	95	480
S.D.	5.89	12.74
C.V. (%)	6.23	2.66

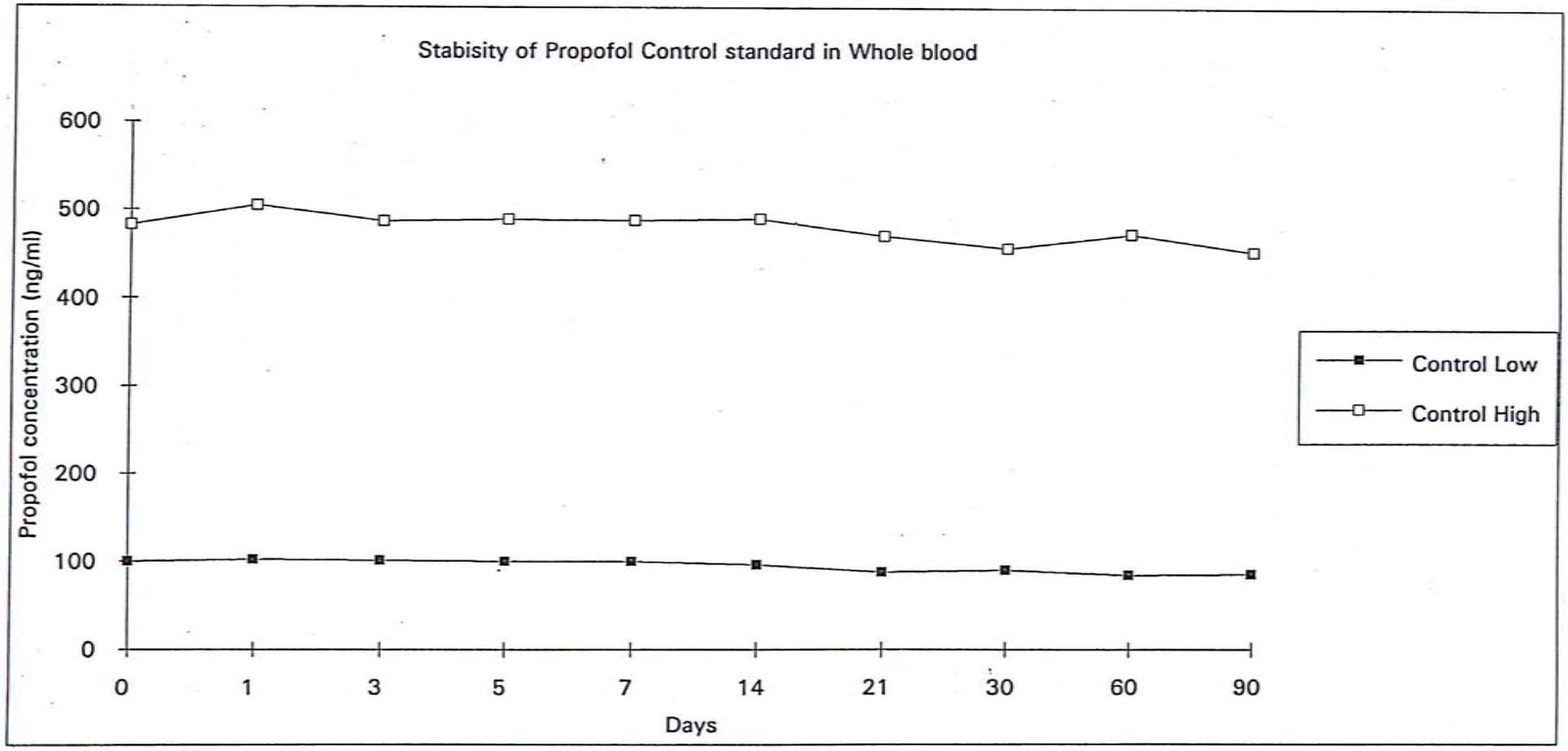


Fig 3-5: Stability of propofol control standard in whole blood

4: Discussions of propofol content analysis

4.1: Calibration standard of propofol.

The previously reported method for propofol using high pressure liquid chromatography (HPLC) and ultraviolet detection of the indophenol derivative (Adam *et al.* 1981) was not considered sufficiently sensitive (limit of quantification ca. 15 ng/ml) for the monitoring of all clinical studies, The combination of HPLC and fluorescence detection has improved the sensitivity of propofol measurements. The method described is more rapid and simpler to use than the indophenol procedure and has an improved limit of quantification of ca. 2 ng/ml (Plummer 1987). Our experimental and analytical method is similar to Plummer's (as described in this chapter **2:** Methods for propofol content analysis) except the following points.

a) In our procedure, blood samples were collected in heparinizied tubes and **0.5 ml** of blood were taken each time, 50 μl of internal standard solution was added to each sample. Plummer's blood samples were collected in oxalated tubes and 1 ml was taken each time. with 20 μl internal standard solution diluted to an approximate working concentration was added to each sample).

b) The HPLC mobile phase consisted of 660 ml acetonitrile 340 ml Mill-Q water (containing 3.4 mL Glacial acetic acid). Plummer's mobile phase consisted of 600 ml of acetonitrile , 400 ml of distilled water and 1 ml of trifluoroacetic acid.

c) Our solvent flow rate was 1.7 ml/min and 700 psi operating pressure, while Plummer's flow rate was 1.5 ml/min.

The method was linear from 2 ng/ml to 3000 ng/ml. The concentration of propofol in standard (50, 100, 250, 500, 1000, 3000 ng/ml) and control (100, 500 ng/ml) samples were calculated using the regression parameters obtained from the calibration graph.

In some instances, we used plasma as sample matrix for preparing the calibration standards instead of whole blood due to the unavailability of the whole blood. We compared the results of calibration standards prepared using either whole blood or plasma as sample matrix and found that there was no significant difference in the value of the slope between the two matrixes. (as described **Table 3-7:** Calibration of propofol standard in whole blood & in plasma, and **Fig. 3-6:** Comparison of results obtained from the calibration of propofol standard in whole blood & plasma)

Table 3-6:

Regression parameters of Calibration of

Propofol standard & Control standard in Whole blood

	Constant	Std Err of Y Est	R Squared	No. of Observations	Degrees of Freedom	X Coefficient(s)	Std Err of Coef.	Factor
C-1A	-0.08188	0.16126	0.99679	6	4	0.00226	0.00006	442
C-1B	0.00850	0.07348	0.97598	10	8	0.00209	0.00012	478
C-2A	-0.03894	0.16457	0.99484	18	16	0.00210	0.00004	476
C-2B	0.04212	0.02389	0.99721	9	7	0.00200	0.00004	500
C-2C	-0.00088	0.05255	0.99668	5	3	0.00203	0.00007	493
C-2D	-0.02371	0.06061	0.99585	5	3	0.00209	0.00008	478
C-2E	-0.01642	0.02047	0.99948	5	3	0.00201	0.00003	498
C-3A	0.00585	0.07834	0.99854	12	10	0.00182	0.00002	549
C-3B	-0.04347	0.08848	0.99037	5	3	0.00200	0.00011	500
C-3C	-0.06998	0.07616	0.99378	5	3	0.00215	0.00010	465
C-3D	-0.08203	0.10449	0.98819	5	3	0.00213	0.00013	469
C-4A	-0.00982	0.12595	0.99637	18	16	0.00192	0.00003	521
C-4B	-0.01870	0.04450	0.99716	5	3	0.00186	0.00006	538
C-4C	-0.03411	0.03736	0.99803	5	3	0.00188	0.00005	532
C-4D	0.09176	0.20722	0.93915	5	3	0.00183	0.00027	546
C-4E	0.00263	0.01058	0.99981	5	3	0.00173	0.00001	578
C-4F	-0.01814	0.01861	0.99946	5	3	0.00178	0.00002	562
C-4G	-0.00511	0.01073	0.99982	5	3	0.00181	0.00001	552
C-5A	0.01243	0.10112	0.99769	18	16	0.00193	0.00002	518
C-5B	-0.01156	0.03471	0.99831	5	3	0.00188	0.00004	532
C-5C	0.02740	0.04682	0.99681	5	3	0.00185	0.00006	540
C-5D	0.02417	0.03839	0.99788	5	3	0.00186	0.00005	541
n	22	22	22			22	22	22
Mean	-0.01090	0.07183	0.99310			0.00196	0.00006	514
S.D.	0.03922	0.05263	0.01284			0.00014	0.00006	35.36947
C.V.(%)	-359.72	73.26457	1.29289			6.98415	87.23303	6.881221

Table 3-7:

Calibration of Propofol Standard in Whole blood & Plasma

Whole blood					Plasma				
ng/ml	Ratio	Mean	S.D.	C.V. (%)	ng/ml	Ratio	Mean	S.D.	C.V. (%)
50A	0.11				50A	0.10			
50B	0.11	**0.11**	0.0041	3.66	50B	0.12	**0.11**	0.0089	8.33
50C	0.12				50C	0.10			
100A	0.23				100A	0.20			
100B	0.21	**0.22**	0.0089	4.10	100B	0.23	**0.21**	0.0111	5.21
100C	0.21				100C	0.21			
250A	0.41				250A	0.46			
250B	0.44	**0.45**	0.0289	6.47	250B	0.51	**0.50**	0.0267	5.33
250C	0.49				250C	0.53			
500A	0.95				500A	0.94			
500B	0.97	**0.96**	0.0089	0.93	500B	0.99	**0.98**	0.0267	2.72
500C	0.95				500C	1.01			
1000A	2.24				1000A	1.92			
1000B	2.03	**2.08**	0.1044	5.01	1000B	2.13	**2.03**	0.0711	3.51
1000C	1.98				1000C	2.03			
3000A	6.24				3000A	5.93			
3000B	5.85	**6.26**	0.2911	4.65	3000B	6.03	**5.93**	0.0711	1.20
3000C	6.70				3000C	5.82			

Regression Output:		Regression Output:	
Constant	-0.0333	Constant	0.01335
Std Err of Y Est	0.16406	Std Err of Y Est	0.05938
R Squared	**0.99486**	R Squared	**0.99924**
No. of Observations	18	No. of Observations	18
Degrees of Freedom	16	Degrees of Freedom	16
X Coefficient(s)	0.0021	X Coefficient(s)	0.00197
Std Err of Coef.	3.8E-05	Std Err of Coef.	1.4E-05

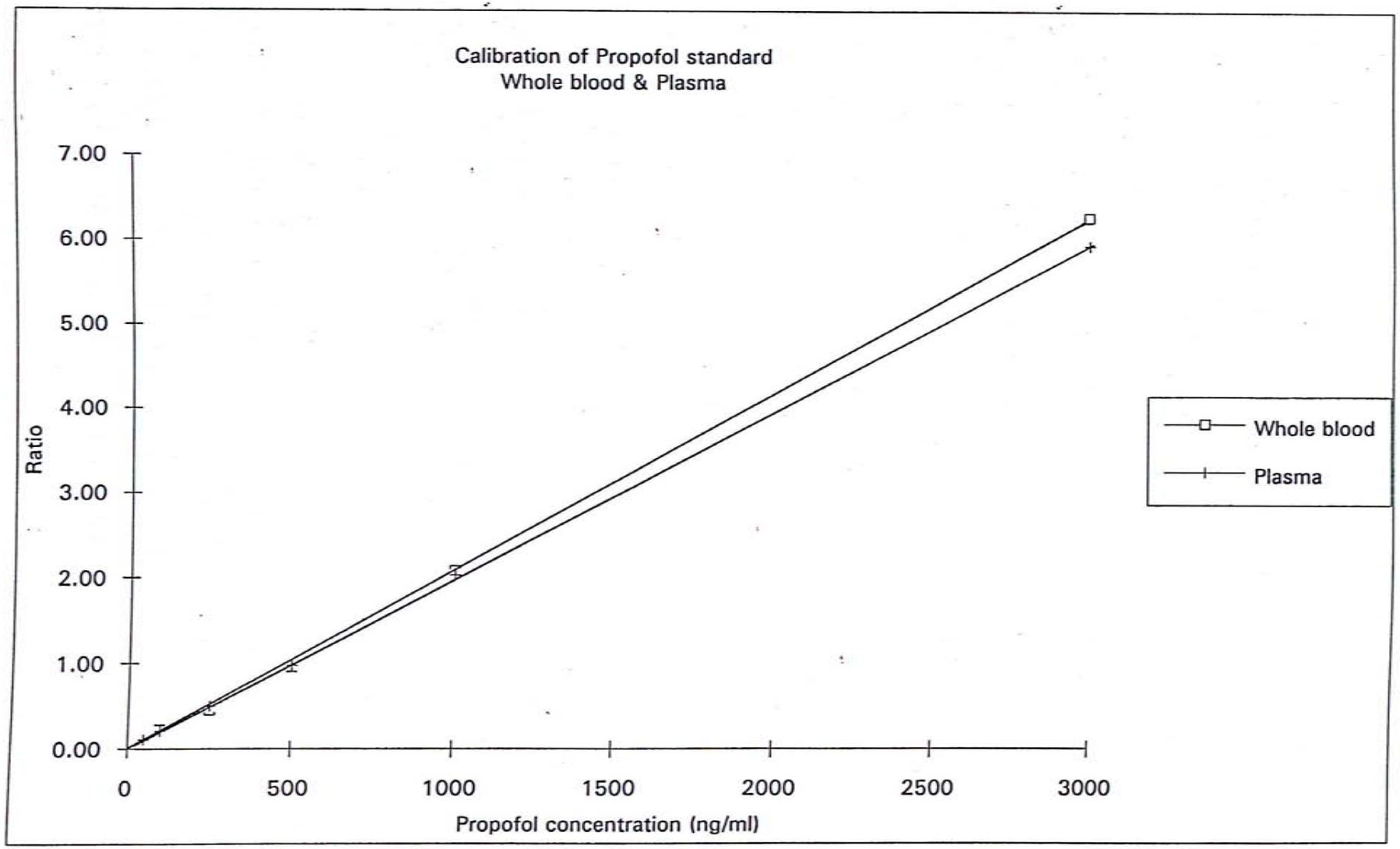


Fig 3-6; Comparison of results obtained from the calibration of Propofol standard in Whole blood Plasma

Table 3-8: Comparison of results obtained from the calibration of propofol by our methods and plummer's (1987)

	Calibration range (ng/ml)	Mean correlation coefficient
Our results (n=22)	0-3000	0.9931
Plummer's results (n=10)	0-6000	0.9993

Table 3-9: Comparison of results obtained from the between-batch coefficient of variation by our method and plummer's (1987)

Our results			Plummer's results		
Mean (ng/ml)	S.D. (ng/ml)	C.V. (%)	Mean (ng/ml)	S.D. (ng/ml)	C.V. (%)
50	0.0104	9.7850	50	0.004	8.0
100	0.0178	8.5220	34	0.003	8.8
250	0.0398	8.1776	57	0.007	12.3
500	0.0488	5.1353	1710	0.05	2.9
1000	0.0808	4.2039	4110	0.14	3.4
3000	0.4602	7.6469	5920	0.26	4.4

Although the limit of detection in the method used by Plummer is similar to our method which is 2 ng/ml, we have the advantage of detecting it using only 0.5 ml of sample as against 1 ml which was used in his method. The coefficient of variations are similar.

4.2: Precision and accuracy of analytical method.

Precision and accuracy determine the error of analysis and are the most important criteria for judging the performance of an analytical method. Normally, precision refers to the variation of scatter of the measurements about the mean value. Some analysts use the terms reproducibility and repeatability instead to refer to the scattering of the assay results obtained by the same method but under different and same conditions (*i*.e. operator, laboratory, instrument, time), respectively. For analysis of drug and other substances in biological samples for pharmacokinetic evaluation, precision and accuracy are most often estimated by analysis of replicate seeded control samples at several concentration levels, preferably three or more, over the expected concentration range of the samples. The precision of the analysis is estimated as the relative standard deviation (coefficient of variation) of the measured concentrations of replicate samples and the accuracy is estimated as the percent differences (bias) between the mean values and true or known concentrations. When the analysis of the seeded control samples are carried out in the same assay run or on the same day the precision data are reported as within-day precision as compared with between-day precision for which the analysis is performed on different days. The criteria for acceptable precision and accuracy of the assay results cannot be generalized

because both precision and accuracy depend on the concentrations of the analyses being evaluated (Thompson 1988) and the acceptable criteria depend on the purpose of the analysis. In the analysis of biological samples for pharmacokinetic studies, assay results with <10% relative standard deviation and <10% bias may be considered acceptable (Geoffrey & Win. 1990).

In our method, the intraassay (n = 5) of propofol in whole blood, which is done on one analysis day, was 6.24% at 100 ng/ml and 6.10% at 500 ng/ml (described in **Table 3-3:** Intraassay coefficient of variance and recovery for propofol in whole blood). The interassay (n = 10) for propofol in whole blood, which was done on ten analysis days, was 3.18% at 100 ng/ml and 1.81% at 500 ng/ml (described in **Table 3-4:** Interassay coefficient of variance for propofol in whole blood). The assay results may thus be considered acceptable.

4.3: Extraction efficiency of analytical method.

An initial isolation of drug and metabolites in biological samples by means of extraction, protein precipitation or other procedures is usually performed before these samples are chromatographed to separate the drug, metabolites and the internal standard. Sometimes, a combination of multiple isolation procedures is necessary

in order to allow adequate chromatographic separation. Many of these sample preparation procedures cause a loss of the drugs or metabolites due to incomplete extraction, adsorption, volume loss or co-precipitation. It is seldom that the drugs can be completely recovered from the biological samples, but the reduced recovery should be minimized by optimizing the conditions for the isolation procedures.

Recovery should also be reproducible. An assay method with low but reproducible recovery may be acceptable when the samples are analyzed with appropriate calibration standards. In some literature reports, the concentration of drug found in seeded control samples expressed as the percentage of the known drug concentration is called "recovery".

Like precision and accuracy, recovery may be concentration-dependent and needs to be evaluated over the range of the expected concentration in the samples. However, good precision and accuracy cannot be obtained when the recovery of the analyses is not reproducible.

In some literature reports, the concentration of drug found in seeded control samples expressed as the percentage of the known drug concentration is called "recovery". By this definition, recovery is the same as accuracy and this is the reason that accuracy is reported as "recovery" by some analysts. As defined, the recovery should be close to 100%. However, recovery experiments are usually done when

the assay method is being developed or validated. The purpose is to obtain an overall high recovery of drugs, preferably >75%, from the samples taken through the complete analytical procedures. Accuracy, on the other hand, is continuously monitored as the samples are analyzed.

In our method, the extraction recovery results obtained (n = 5) for propofol in whole blood is 104% at 100 ng/ml and 99% at 500 ng/ml (described in **Table 3-3:** Intraassay coefficient of variance and recovery for propofol in whole blood).

Our results are thus acceptable.

4.4: Stability of analytical method.

Many drugs and their metabolites are relatively unstable and may degrade in the biological samples during the collection and processing of the fresh samples and the subsequent storage, preparation and analysis of the samples. Factors affecting the stability of drugs in biological samples are many and should be evaluated based on each individual drug. In most pharmacokinetic studies the biological samples collected are seldom analyzed immediately after collection, but are stored for later analysis. In our laboratory, the samples were assayed within one month. Long-term storage enhances the

probability of degradation of the drugs and metabolites in the biological samples. The analysis of biological samples should be completed before the degradation of the drugs occurs. Care must be taken to assure that the measured drug concentrations are meaningful and not interfered with by sample degradation and other artifacts due to the procedures for the collection, processing, storage and analysis of the samples.

Stability of whole blood samples are checked by assaying aliquots of a pooled control on separate analysis days (n = 10 up to 3 months for whole blood). A slight decrease in the amount of propofol was detected after 30 days. Hence, we assay all the samples within one month. (**Fig 3-5:** Stability of propofol control in whole blood)

CHAPTER FOUR:

ANALYTICAL TECHNIQUE:

PROTEIN BINDING OF PROPOFOL

1: Introduction protein binding of propofol

Drug disposition. *i*.e. absorption, distribution, metabolism and elimination are the major determinants of the pharmacokinetic profile of any compound. The pharmacological response obtained is, for the majority of drugs, related to the concentration of drug at its receptor site(s). The disposition and ultimate biological effect of the drug may be said to be 'controlled' by the extent of plasma protein binding, since this is the factor limiting the amount of drug available to leave the vasculature and gain access to receptors, tissues and the various sites of elimination and metabolism. As a result, any significant alteration in plasma protein binding can lead to an altered drug distribution and unexpected pharmacological responses. A further consequence of a change in protein binding also

has implication on therapeutic drug monitoring, i.e. alteration of a dose according to an individual's plasma concentration.

At present, two separation methods are widely used: ultrafiltration and equilibrium dialysis.

1.1: Ultrafiltration

In the ultrafiltration of a protein solution containing a ligand, the pressure difference forces the buffer containing free ligand through a permselective membrane. The total volume of ultrafiltrate plus retentate solutions equals the initial solution volume. This method of separation of the bound ligand from the free one is extremely popular because of the easy handling of a large number of samples, as well as the commercial availability of a variety of filtration devices.

1.2: Equilibrium dialysis

In a typical equilibrium dialysis experiment the macromolecular solution is separated by a semi-permeable membrane from the ligand solution. If there is no alteration of ligand or protein(s), the system reaches an equilibrium state known as "steady state". Since, at steady state, the free ligand concentrations are equal on both sides of the dialysis membrane, the concentration detected

in the compartment containing the protein(s) is due to the sum of bound and free drug concentrations, whereas the concentration detected in the other side is that of the free ligand only. Therefore, the concentration of bound ligand is equal to the difference between the total ligand concentrations (bound + free) of the solutions present in each compartment.

Equilibrium dialysis is an ideal approach to studying the binding of small molecules or ions to macromolecules. For example, binding studies are very important in pharmacology. The therapeutic and toxic effects of a drug are directly related to the level of unbound drug in the blood stream. Drug bound to plasma proteins is pharmacologically not effective, therefore it is necessary to determine the level of bound and unbound drug in the blood to find out the proper dosage. The extent of drug binding varies from one patient to another. Low adsorption is an absolute necessity in binding studies because the unbound species must be freely permeable through the membrane to attain equilibrium. High adsorption leads to false results.

The choice of a particular method depends on the stability of the ligand-protein complex and on the accuracy needed, *i.e.* the bound ligand percentage, the binding parameters or the molecular recognition ability of the protein. Equilibrium dialysis is theoretically the most

accurate way to determine free and bound ligands because the equilibrium is not shifted when aliquots are taken from both sides of the dialysis membrane.

Therefore, equilibrium dialysis was adopted as our test procedure.

2: Methods of protein binding of propofol

Refer Appendix for source & manufacturer of all reagents and equipment.

2.1: Material & Solution

2.1.1: Dialysis buffer

Sorensen's phosphate buffer was prepared by mixing 200 ml of Potassium dihydrogen phosphate, M = 136.09 g/mol with 800 ml of Di-sodium hydrogen phosphate, M = 137.99 g/mol and adjusting the pH to 7.4 using Sodium hydroxide solution (1N) or Hydrochloric acid (1N).

2.1.2: Molecularporous Dialysis Membrane

The Spectra/Por regenerated cellulose membrane has proved to be a useful membrane available for laboratory dialysis. It carries no fixed charge and does not absorb most solutes. Hydrophobic microfiltration membranes are often characterized by means of "bubble point" measurements

that give an estimate of pore size. These measurements are not practical for most dialysis membranes because their "pores" are much smaller and because in regenerated cellulose membranes the structure is actually a gel. Instead, the widely used method to characterize dialysis membranes is the molecular weight cut off (MWCO). In this method the permeability of the membrane to a series of molecules of different molecular weights in solution is measured in dialysis experiments. That solute molecular weight that is prevented from permeating (retained) to an extent of 90% is the MWCO. Spectrum uses this method to characterize its dialysis membranes.

Another method that is also used at Spectrum is the measurement of the rate of dialysis. This is carried out by placing a solution with a known concentration of a selected permeable molecular species on one side (retentate side) of the membrane and pure solvent on the other (diffused side). Both are well mixed and the solvent is changed frequently so that the concentration of solute is always negligible. Under these circumstances, the rate of passage of the solute follows first order kinetics, *i.e.*, a plot of the logarithm of concentration in the retentate versus time follows a straight line.

To prevent possible absorption of propofol from the blood sample into the membrane, immerse the Molecularporous dialysis membrane into the Mill-Q water for 24 hours in 4°C and then discard the water. Add 250 ml Mill-Q water and 100

μl of propofol (100 mcg/ml) and mix for one hour at room temperature using a magnetic bar on the stir plate. After one hour, discard the drug solution and wash the membrane ten times using 250 ml portions of Milli-Q water, discarding the washing each time. Finally immerse the membrane into 250 ml Sorensen's phosphate buffer (as described this chapter **2.1.1:** Dialysis buffer) store at 4°C prior to use.

2.2: Equilibrium dialysis

Equilibrium dialysis was performed using a Spectrum equilibrium Dialyser. Drug-containing plasma samples or protein solutions (1 ml) were dialysed against drug-free Sorensen's phosphate buffer (1 ml; pH 7.4) in teflon dialysis chambers separated by Spectrapor dialysis membrane (As described this Chapter **2.1.2:** Molecularporous Dialysis Membrane). The effect of drug concentration on protein binding was determined within the range of 0.25-3.00 μg/ml of Propofol (the usual therapeutic plasma concentration range of the drug). Propofol concentrations in samples were assayed by a high-pressure liquid chromatography method as described above **Chapter Three 2.6:** Procedure. Protein binding was calculated using the formula:

$(C_p - C_{bu})/C_p \times 100\%$

C_p = concentration in plasma after dialysis

C_{bu} = concentration in buffer after dialysis

2.3: Determine the optimum dialysis time to reach equilibrium

The time required for dialysis for each individual ligand must be determined experimentally. Starting with specific ligand concentration, a defined operating temperature and a buffer system, dialysis is measured as a function of time.

2.3.1: Material

A. Propofol (3000 ng/ml) was spiked onto blank plasma to give a concentration of 3000 ng/ml. The mixture was mixed for one hour at room temperature and then store at 4°C prior analysis.

B. Sorensen's phosphate buffer (as described this chapter **2.1.1:** Dialysis buffer).

C. Spectra/Por 2 membranes (Part No's 132678 and 132680)

D. 1 ml syringes equipped with 3 inch blunt nose 22G needles.

E. Teflon cells.

2.3.2: Procedures

A. Fill the compartments on the left side with plasma (containing 3000 ng/ml propofol) and the compartments on the right side with the Sorensen's phosphate buffer.

B. Rotate the Teflon cells at 15 rpm at 37°C.

C. Empty the cells in accordance with the following schedules.

Cell No. 1: after 15 minutes

Cell No. 2: after 30 minutes

Cell No. 3: after 60 minutes

Cell No. 4: after 90 minutes

Cell No. 5: after 120 minutes

Cell No. 6: after 150 minutes

Cell No. 7: after 180 minutes

Cell No. 8: after 210 minutes

D. After reaching equilibrium dialysis, collect the plasma and buffer separately using a 1 ml syringe with 3 inch blunt nose 22G needles transfer to 1.5 ml eppendorf tubes.

E. According **Chapter Three 2.6:** Procedure to analysis propofol concentration.

F. Finally, binding parameter are calculated.

G. Each experiment was done in duplicate.

3: Results of protein binding of propofol

3.1: Results for optimum dialysis time to reach equilibrium

Described as **Table 4-1:** Ligand binding experiments result

Table 4-1:

Ligand binding experiments result

Time	Buffer				Plasma			
(mins)	Label	Ratio	Propofol (ng/ml)	Bound (%)	Label	Ratio	Propofol (ng/ml)	Bound (%)
15	B1	0.0298	10.96	0.53	P1	5.1740	2064.41	99.47
	B2	0.0475	18.97	0.60	P2	7.9284	3163.65	99.40
30	B1	0.0975	38.92	1.32	P1	7.3873	2947.54	98.68
	B2	0.1182	47.17	1.72	P2	6.8668	2739.86	98.28
60	B1	0.1160	46.28	1.56	P1	7.4476	2971.59	98.44
	B2	0.1045	41.70	1.66	P2	6.2841	2507.34	98.34
90	B1	0.2224	88.74	3.10	P1	7.1710	2861.23	96.90
	B2	0.1342	53.55	1.82	P2	7.3755	2942.82	98.18
120	B1	0.1470	58.66	2.24	P1	6.5651	2619.49	97.76
	B2	0.1377	54.93	2.51	P2	5.4942	2192.20	97.49
150	B1	0.1439	57.43	2.20	P1	6.4927	2590.59	97.78
	B2	0.1483	59.17	2.30	P2	6.4273	2564.49	97.69
180	B1	0.1258	50.21	1.88	P1	6.7028	2674.41	98.12
	B2	0.1314	52.43	2.37	P2	5.5485	2213.86	97.63
210	B1	0.1378	54.98	2.15	P1	6.4220	2562.38	97.85
	B2	0.1335	53.27	2.03	P2	6.5880	2628.61	97.97

3.2: Results for reproducibility

Pooled control plasma is spiked with known concentration of propofol (3000 ng/ml) stored at 4°C. These pooled controls were assayed for propofol content on Day 0, 1, 3, 5, 7, 14, 30. (6 trials each day). Some of these pooled controls were dialysed and the propofol content in both plasma/buffer compartments determined. (5 trials each day).

3.2.1: Intraassay coefficient of variation (One analysis day) of propofol in plasma and in protein binding

The intraassay coefficient of variation (n = 6) of total propofol in plasma, which is done on one analysis day, was 1.97% at 3000 ng/ml, and protein bound propofol was 0.11% and free propofol 3.65% (described in **Table 4-2:** Intraassay coefficient of variance for propofol in plasma **& Table 4-3:** Intraassay coefficient of variance for propofol in protein binding)

3.2.2: Interassay coefficient of variation (Seven analysis days) of propofol in plasma and in protein binding

The interassay coefficient of variation (n = 42) of total propofol in plasma is 2.19% at 3000 ng/ml (described in **Table 4-4:** Interassay coefficient of variance for propofol in plasma), protein bound propofol was 0.23% and free propofol was 8.57% (described in **Table 4-5:** Interassay coefficient of variance for propofol in protein binding).

Table 4-2:

Intraasay coefficient of variance for Propofol in Plasma

Day	Sample	Concentration (ng/ml)
1	Sample-1	2876
	Sample-2	2933
	Sample-3	2979
	Sample-4	2788
	Sample-5	2811
	Sample-6	2904
Mean (n = 6)		2882
S.D.		56.91
C.V. (%)		1.97

Table 4-3:

Intraasay coefficient of variance for Propofol in Protein binding

Day	Sample	Buffer	Plasma	Bound	Free
		Conc. ng/ml	Conc. ng/ml	(%)	(%)
1	Sample-1	71.39	2545	97.19	2.81
	Sample-2	75.05	2681	97.20	2.80
	Sample-3	73.15	2545	97.13	2.87
	Sample-4	71.35	2692	97.35	2.65
	Sample-5	77.41	2529	96.94	3.06
Mean (n = 5)				97.16	2.84
S.D.				0.10	0.10
C.V. (%)				0.11	3.65

Table 4-4:

Interassay coefficient of variance for Propofol in Plasma

Day	0	1	3	5	7	14	30
	Conc. (ng/ml)	Conc. (ng/ml)	Conc. (ng/ml)	Conc. (ng/ml)	Conc. (ng/ml)	Conc. (ng/ml)	Conc. (ng/ml)
Sample-1	2947	2876	2773	2894	2795	2902	2832
Sample-2	2881	2933	2793	2873	2805	3069	2755
Sample-3	2898	2979	2893	2942	2942	2961	2884
Sample-4	2876	2788	2805	2952	2857	3051	2846
Sample-5	2956	2811	2879	2974	2807	3037	2872
Sample-6	2982	2904	2807	2923	2807	3027	2912
Mean (n = 42)	2893						
S.D.	63.29						
C.V. (%)	2.19						

Table 4-5:

Interassay coefficient of variance for Propofol in Protein binding

Day	Sample	Buffer Conc. ng/ml	Plasma Conc. ng/ml	Bound (%)	Free (%)
0	Sample-1	80.54	2559	96.85	3.15
	Sample-2	82.7	2750	96.99	3.01
	Sample-3	73.48	2573	97.14	2.86
	Sample-4	72.28	2642	97.26	2.74
	Sample-5	70.16	2782	97.48	2.52
1	Sample-1	71.39	2545	97.19	2.81
	Sample-2	75.05	2681	97.20	2.80
	Sample-3	73.15	2545	97.13	2.87
	Sample-4	71.35	2692	97.35	2.65
	Sample-5	77.41	2529	96.94	3.06
3	Sample-1	69.37	2619	97.35	2.65
	Sample-2	65.58	2599	97.48	2.52
	Sample-3	65.12	2574	97.47	2.53
	Sample-4	67.77	2658	97.45	2.55
	Sample-5	67.13	2796	97.60	2.40
5	Sample-1	62.21	2311	97.31	2.69
	Sample-2	60.14	2621	97.70	2.30
	Sample-3	65.09	2486	97.38	2.62
	Sample-4	59.14	2457	97.59	2.41
	Sample-5	66.53	2574	97.42	2.58
7	Sample-1	57.7	2255	97.44	2.56
	Sample-2	63.67	2418	97.37	2.63
	Sample-3	51.35	2091	97.54	2.46
	Sample-4	62.49	2279	97.26	2.74
	Sample-5	55.41	2468	97.75	2.25
14	Sample-1	60.4	2718	97.78	2.22
	Sample-2	46.78	2470	98.11	1.89
	Sample-3	43.76	2726	98.39	1.61
30	Sample-1	60.12	2223	97.30	2.70
	Sample-2	61.8	2317	97.33	2.67
	Sample-3	62.53	2536	97.53	2.47
	Sample-4	63.66	2477	97.43	2.57
	Sample-5	56.68	2620	97.84	2.16
	Mean (n =33)			97.44	2.57
	S.D.			0.22	0.22
	C.V. (%)			0.23	8.57

3.3: Results for recovery of propofol in plasma and in protein binding

An initial isolation of drug and metabolites in biological samples by means of extraction, protein precipitation or other procedures is usually performed before these samples are chromatographed to separate the drug, metabolites and the internal standard. Sometimes, a combination of multiple isolation procedures is necessary in order to allow adequate chromatographic separation. Many of these sample preparation procedures cause a loss of the drugs or metabolites due to incomplete extraction, adsorption, volume loss or co-precipitation. It is seldom that the drugs can be completely recovered from the biological samples, but the reduced recovery should be minimized by optimizing the conditions for the isolation procedures.

3.3.1: Recovery of propofol (unheated samples)

Recovery may be concentration-dependent and needs to be evaluated over the range of the expected concentrations in the samples. Pooled control plasma is spiked with a known concentration of propofol (3000 ng/ml). This pool is stored in -70°C and then assayed for propofol content on Day 0, 1, 3, 5, 7, 14, 30. (6 trials each day). The extraction recovery results obtained for all 7 analysis days (n = 42) for propofol in plasma is 96.42% and S.D.

1.70 (described in **Table 4-6:** Recovery of propofol in plasma).

3.3.2: Recovery of propofol (samples heated at 37°C)

The same lot of pooled plasma spiked with propofol (3000 ng/ml) as mentioned in the previous paragraph is used. This time however, the samples are heated in a 37°C water bath for 4 hours and then assayed for propofol content. This is done on three separate occasions (2 trials each day). The results obtained is compared with the results of those samples which were not heated to 37°C prior to propofol assay on the same analysis day. (Day 5, 14, 30). The percentage recovery of heated (n = 2) against unheated (n = 6) samples is 90.64% and S.D. 0.72, [described in **Table 4-7:** Recovery of heated (37°C) & unheat propofol in plasma].

3.3.3: Recovery of propofol (after dialysis at 37°C)

Pooled control plasma spiked with known concentration of propofol (3000 ng/ml) is dialysed at 37°C water bath and after 4 hours of dialysis, the plasma and buffer compartments and are then assayed for propofol content. The same lot of pooled samples as mentioned above is used. This study is done on three separate occasions (Day 5, 14, 30) and two trials are done per day. The percentage recovery results obtained for all 3 analysis days (n = 6) for propofol in plasma is 97.15% and S.D. 1.11 [described in

Table 4-8: Recovery of heated (37°C) dialysed & not dialysed for propofol in plasma].

Table 4-6:

Recovery of Propofol in Plasma

Day	Expect Conc. (ng/ml)	Sample Conc. (ng/ml)	Recovery (%)
0	3000	2923	97.43
1	3000	2882	96.07
3	3000	2825	94.17
5	3000	2926	97.53
7	3000	2835	94.50
14	3000	3008	100.27
30	3000	2850	95.00
Mean (n = 42)			96.42
S.D.			1.70
C.V. (%)			1.77

Table 4-7:

Recovery of Heated (37°C) & Unheated Propofol in Plasma

Day	Sample Concentration (ng/ml)		Recovery
	Unheated	Heated	(%)
	Mean (n = 6)	Mean (n = 2)	
5	2926	2621	89.58
14	3008	2726	90.63
30	2850	2614	91.72
Mean (n = 6)			90.64
S.D.			0.72
C.V. (%)			0.79

Table 4-8:

Recovery of heated (37°C)

Dialyzed & Not dialyzyed Propofol in Plasma

Day	Sample concentration (ng/ml)		Recovery
	Not Dialyzed	Dialyzed	(%)
	Mean (n = 2)	Mean (n = 5)	
5	2621	2552	97.37
14	2726	2688	98.61
30	2614	2496	95.49
Mean (n = 6)			97.15
S.D.			1.11
C.V. (%)			1.14

3.4: Results for stability of propofol in plasma

In our laboratory, the samples were assayed within one month. Long-term storage enhances the probability of degradation of the drugs and metabolites in the biological samples. The analysis of biological samples should be completed before the degradation of the drugs occurs.

Pooled plasma is spiked with known volume of standard Propofol to prepare pooled controls containing 3000 ng/ml. Aliquots of 0.7 ml of this pooled control are transferred into 1.5 ml eppendorf tube and stored at 4°C to be subsequently assayed on separate analysis to check for stability during storage. The graph bases on the **Table 4-6:** Recovery of propofol in plasma (**Fig 4-2:** Stability of propofol control in plasma)

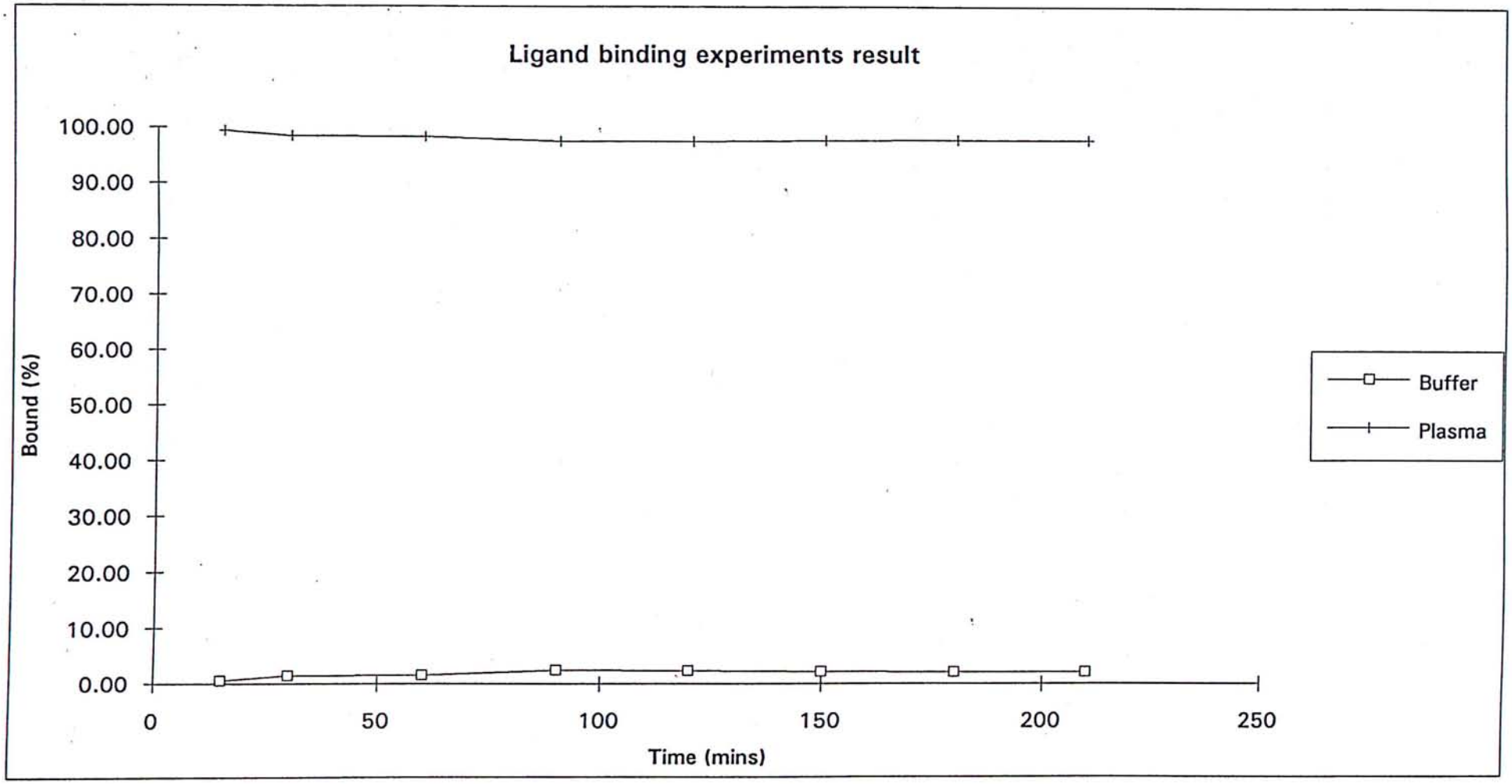


Fig 4-1: Ligand binding experiments result

4: Discussions of protein binding of propofol

4.1: Optimum dialysis time to reach equilibrium

Because ligand binding has an optimum dialysis time, hence the need to determine a more reliable equilibrium time. Based on **Table 4-1:** and **Fig 4-1:** Ligand binding experiments result, this optimum dialysis time is between 120 to 210 minutes. No significant change in the amount of bound was detected from 120 min to 210 min. We decided to choose 240 minutes to assure that equilibrium was achieved.

4.2: Discussion for Intraassay & Interassay coefficient of variation of propofol in plasma and in protein binding

The precision of the analysis is estimated as the relative standard deviation (coefficient of variation) of the measured concentrations of replicate samples and the accuracy is estimated as the percent differences (bias) between the mean values and true or known concentrations. When the analysis of the seeded control samples are carried out in the same assay run or on the same day the precision data are reported as within-day precision as compared with between-day precision for which the analysis is performed on different days. The criteria for acceptable precision and accuracy of the assay results cannot be generalized

because both precision and accuracy depend on the concentrations of the analyses being evaluated (Thompson 1988) and the acceptable criteria depend on the purpose of the analysis. In the analysis of biological samples for pharmacokinetic studies, assay results with <10% relative standard deviation and <10% bias may be considered acceptable (Geoffrey & Win. 1990).

In our method, the intraassay coefficient of variance of total propofol in plasma (n = 6), which was done on one analysis day, was 1.97% at 3000 ng/ml, and protein bound propofol was 0.11% and free propofol was 3.65% (described in **Table 4-2:** Intraassay coefficient of variance for propofol in plasma & **Table 4-3:** Intraassay coefficient of variance for propofol in protein binding). The interassay coefficient of variation of total propofol in plasma (n = 42) was 2.19% at 3000 ng/ml (described in **Table 4-4:** Interassay coefficient of variance for propofol in plasma), protein bound propofol was 0.23% and free propofol was 8.57% (described in **Table 4-5:** Interassay coefficient of variance for propofol in protein binding). The assay results may be considered acceptable.

4.3: Recovery of propofol in plasma and in protein binding

Recovery should also be reproducible. An assay method with low but reproducible recovery may be acceptable when the samples are analyzed with appropriate calibration standards. In some literature reports, the concentration of drug found in seeded control samples expressed as the percentage of the known drug concentration is called "recovery".

Like precision and accuracy, recovery may be concentration-dependent and need to be evaluated over the range of the expected concentration in the samples. However, good precision and accuracy cannot be obtained when the recovery of the analyses is not reproducible.

In some literature reports, the concentration of drug found in seeded control samples expressed as the percentage of the known drug concentration is called "recovery". By this definition, recovery is the same as accuracy and this is reason that accuracy is reported as "recovery" by some analysts. As defined, the recovery should be close to 100%. However, recovery experiments are usually done when the assay method is being developed or validated. The purpose is to obtain an overall high recovery of drugs, preferably >75%, from the samples taken through the complete analytical procedures. Accuracy, on the other hand, is continuously monitored as the samples are analyzed.

In our method, The extraction recovery results obtained for all 7 analysis days (n = 42) for propofol in plasma is 96.42% and S.D. 1.70 (described in **Table 4-6:** Recovery of Propofol in Plasma).

This time however, the samples are heated in a 37°C water bath and then assayed for propofol content. The results obtained is compared with the results of those samples which were not heated to 37°C prior to propofol assay on the same analysis day. The percentage recovery of heated (n = 2) against unheated (n = 6) samples is 90.64% and S.D. 0.72, (described in **Table 4-7:** Recovery of heated (37°C) & unheat propofol in plasma). Between **Table 4-7:** Recovery of heated (37°C) and unheated propofol in plasma and **Table 4-8:** Recovery of heated (37°C) dialysed and undialysed propofol in plasma, the difference between the results from the two tables is only 6.51%. However, this does not produce a significant effect on the outcome of the ligand binding experiments as shown in **Table 4-1:** Ligand binding results, whereby the total propofol content (buffer + plasma) does not change significantly any more after 120 minutes.

Propofol (3000 ng/ml) is dialysed at 37°C water bath and after 4 hours of dialysis, the plasma and buffer compartments and are then assayed for Propofol content. The same lot of pooled samples as mentioned above is used. The percentage recovery results obtained for all 3 analysis days (n = 6) for propofol in plasma is 97.15% and S.D.

1.11, (described in **Table 4-8:** Recovery of heated (37°C) dialysed & not dialysed propofol in plasma).

Above results of recovery may be acceptable, because the recovery close to 100%.

4.4: Discussion for stability of propofol in plasma

Stability of plasma samples are checked by assaying aliquots of a pooled control on separate analysis days (n = 8 up to 1 months for plasma). No significant decrease in the amount of Propofol was detected throughout the course of the stability studies. (**Fig 4-2:** Stability of propofol control in plasma)

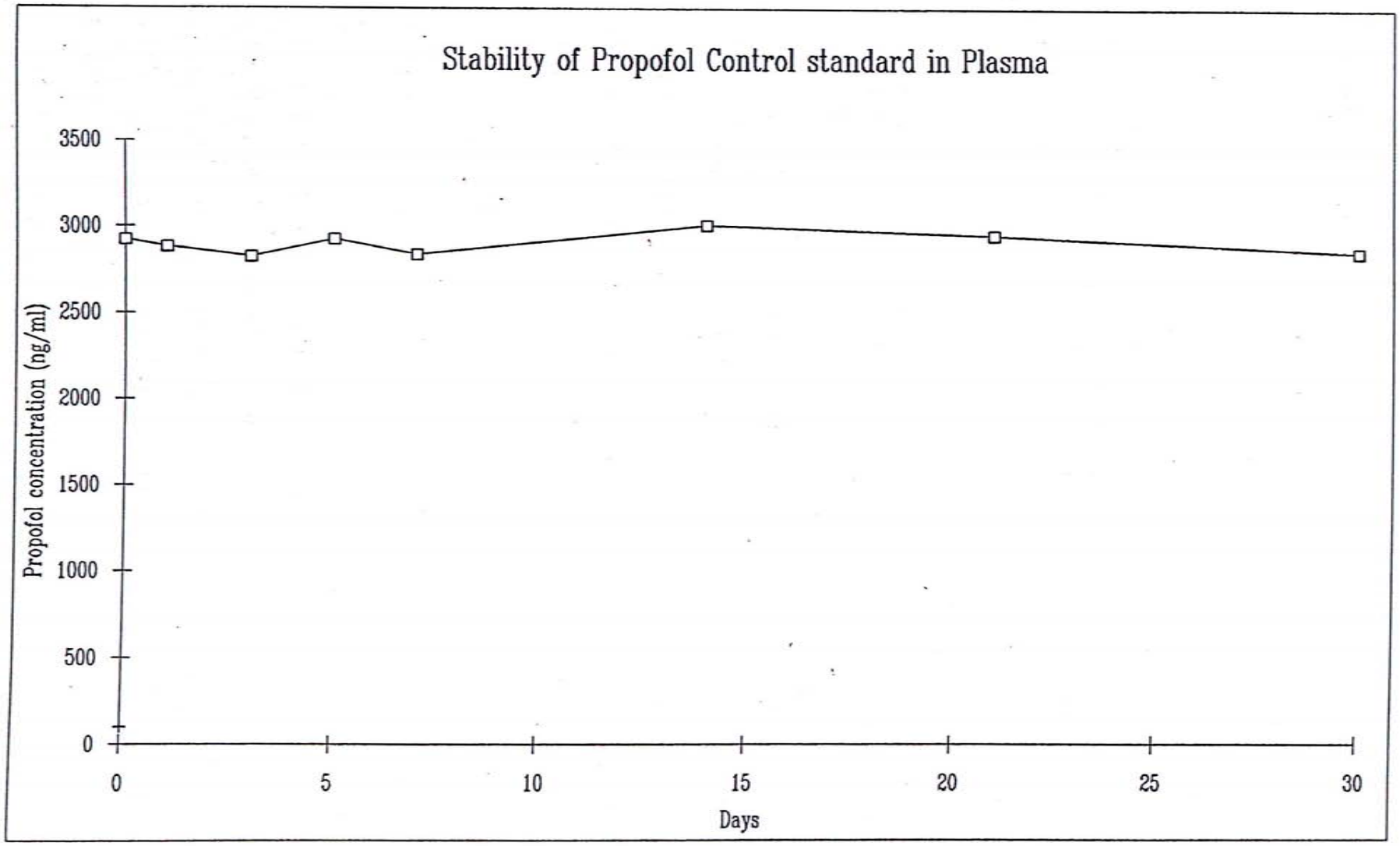


Fig 4-2: Stability of propofol control standard in Plasma

CHAPTER FIVE

CLINICAL APPLICATION

OF PHARMACOKINETIC STUDIES

1: Introduction pharmacokinetic model controlled infusion

The following infusion study is an example of the application of our method of analysis in children: A prospective evaluation of pharmacokinetic model controlled infusion of propofol in paediatric patients (Short *et al*, 1994).

1.1: Theoretical basis

The disposition of infused propofol is assumed to be described by an equation representing a three-compartment model, with drug entering or leaving the system only by the first compartment (**Fig. 5-1:** The three-compartment pharmacokinetic model)

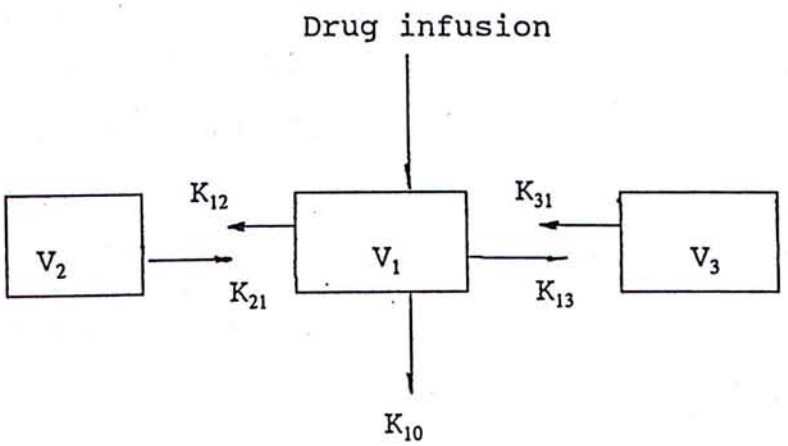


Fig. 5-1: The three-compartment pharmacokinetic model where V_1, V_2, and V_3 represent the volumes of distribution. V_1 is the initial volume of distribution. The intercompartmental and elimination rate constants are shown.

The blood concentration = concentration in the central compartment (V_1).

Initially, all compartments have propofol concentrations of zero. To achieve the target blood concentration rapidly (volume 1 concentration), sufficient drug is given as an initial bolus dose.

$$\text{Dose or } A_1 = C_{TG} * V * Wt$$

If the blood concentration (i.e. that in volume 1) is to be

kept constant, the amount of drug entering it and leaving it must be equal. Drug leaves the blood to pass into volumes 3 and volumes 2 at a gradually decreasing rate as the concentrations in these compartments increase. Drug also leaves the blood because it is metabolized, given by (k_{10} * volume 1) ml/min.

If we consider only the transfer of drug from one compartment to an adjacent compartment, the rate of drug transfer equals the amount of drug in the source compartment times a rate constant, k, to which we append a subscript indicating which compartment is involved. Of course, drug is transferring in the opposite direction as well at a rate that equals the amount in adjacent compartment times a different rate constant. The net rate of change in drug concentration in a compartment is the rate at which the drug is entering the compartment, less the rate at which the drug is leaving the compartment.

Theoretically, it would take an infinite time for the peripheral compartments to achieve the target concentration. For the slowest compartment to achieve at least 94% of any target concentration, it could take approximately 16 hours (Tackley *et al*, 1989). Finally, when there is no further redistribution to other compartments, the amount of drug to be infused would be equal to that being cleared.

The rate of change in each compartment for a 3-

compartment model can be described with the following equations (Maitre & Shafer, 1990).

$$dA_1/dt = k_{21}A_2 + k_{31}A_3 - (k_{10} + k_{12} + k_{13}) * A_1 \qquad (1)$$

Where $A_2/dt = A_1k_{12} - A_2k_{21}$

$A_3/dt = A_1k_{13} - A_3k_{31}$

where A_1, A_2, and A_3 are the amount of drug in compartments V_1, V_2, and V_3, respectively. These equations cannot be used directly by a calculator, because dt is an infinitely small time interval. However, we can approximate these equations by substituting Δt for dt, and assigning a small time interval, (e.g., 10 s), to Δt. This is known as "Euler's" Numeric Integration technique. We can thus approximate the change in drug concentration in each compartment with the following equations:

$$\Delta A_1 = [k_{21}A_2 + k_{31}A_3 - (k_{10} + k_{12} + k_{13})A_1] * \Delta t \qquad (2)$$

Equation (2) can be easily modified to account for a bolus injection (amount = B) and a continuous infusion (rate = R) of drug to the central compartment:

$$\Delta A_1 = [k_{21}A_2 + k_{31}A_3 - (k_{10} + k_{12} + k_{13})A_1 + R] * \Delta t + B \qquad (3)$$

Thus a pseudosteady state concentration can be maintained in V_1, the central compartment. Re-write the equation for R, ethan you have the infusion rate by pharmacokinetic model controlled or

$$R = | A_1 - A_1' | \qquad (4)$$

Where A_1' is the next value of A.

1.2: Use of computer & appropriate pump

The propofol dose required to maintain the desired plasma concentration was delivered using an Ohmeda 9000 infusion pump controlled by a 386SX IBM compatible laptop computer connected via an Rs232C serial interface. A program was written in 'C' (Turbo C, Borland, USA) to control the pump (by Y.H. Tam).

2: Results of propofol pharmacokinetic studies

2.1: Sample preparation

Permission to conduct the study was obtained from the Research Ethic Committee of the Faculty of Medicine of the Chinese University of Hong Kong, and informed consent were obtained from the children's parents. Ten patients aged from 4 to 8 years were studied. All were healthy (ASA I) had been full term babies, had no contraindications to the drugs used in the trial and were undergoing minor surgical procedures associated with minimal blood loss. Patients with preoperative haemoglobin level less than 11.5 g/dl were excluded. All the operations were performed in the operation theatres of the Prince of Wales Hospital, (Shatin, New Territories, Hong Kong). EMLA Cream 5% (2.5% lignocaine and 2.5% prilocaine, Astra Pharmaceuticals, Sweden) was applied to the dorsum of one hand one hour preoperatively to assist with intravenous catheter placement. Anaesthesia was induced with intravenous propofol given by an infusion pump and maintained using the propofol infusion and nitrous oxide 70% in oxygen. All patients breathed spontaneously. The propofol infusion model used was similar to that of Marsh *et al*, 1991. The system consisted of an Ohmeda 9000 syringe pump controlled by a 386SX IBM compatible computer via an RS232C serial interface. After having purged the intravenous tubing using

the purge option on the pump, an initial bolus of propofol was given according to body weight at the pump's top rate (1200 ml/h) to rapidly attain the desired plasma concentration. The infusion rate was then adjusted by the computer to maintain the desired plasma concentration using a three compartment pharmacokinetic model. The target plasma concentration was adjusted up or down in 1-2 μg/ml steps as according to standard clinical criteria, including movement in response to pain, respiratory cardiovascular and sympathetic nervous system signs. Time from switching off the infusion until eye opening in response to verbal command was recorded (Short *et al*. 1994).

A second intravenous catheter was placed in a large vein in the opposite limb to that used for the propofol infusion. It was used for taking venous blood samples for later analysis of blood propofol concentrations. Two ml blood samples were drawn at the time of surgical incision and then at approximately ten minutes intervals during the procedure. Up to four samples were also taken in the recovery room during the first hour following termination of the infusion. A maximum of eight blood samples were taken from each patient (Short *et al*. 1994). Blood samples were collected into a 5 ml heparinized tube and stored at 4°C for later analysis using high-pressure liquid chromatography with fluorescence detection. (as described in Chapter Three 2: Methods for propofol content analysis)

Analysis of whole blood concentrations propofol (Cp)

was performed. For specimens with expected propofol concentrations above 3000 ng/ml dilution was used to decrease propofol concentration to within the calibrated range of the assay (detail describe as above **Chapter Three: 3: Calibration standard of propofol).**

2.2: Computer control infusion of propofol according to pharmacokinetic model

Fig 5-2: illustrates the course of a typical anaesthetic administered to a 18.7 kg child (aged 5 years) undergoing circumcision using the computer aided infusion device. In this study we prospectively tested the use of a previously published algorithm for administering propofol to paediatric patients (Marsh *et al*. 1991). Anaesthesia was induced by selecting an initial theoretical target concentration of 10 μg /ml. Thereafter, the target was reduced to 6.00 μg/ml without deterioration in the operating conditions, and then the target concentration was titrated according to the depth of anaesthesia required. The anaesthesist retained at all times the option of increasing or decreasing the theoretical target blood concentration according to his or her clinical impression of the quality of anaesthesia. At the termination of surgery, the pump was switched off and the patient transferred to the recovery room, where the device calculated the theoretical decay of propofol concentration

in the patient's blood until eye opening was observed. In **Fig 5-2:**, the measured values of blood propofol were overlaid on the system record of the theoretical propofol profile and it could be seen that, in this individual, the delivery system consistently overpredicted the measured blood concentration of propofol.

Fig 5-2: Predicted and measured blood concentrations of propofol during anaesthesia in a 18.7 kg patient (Age 5 years). The solid line represents predicted values, measured values are marked x.

Ohmeda 9000 Syringe Pump Operation Result (Filename : PROP10

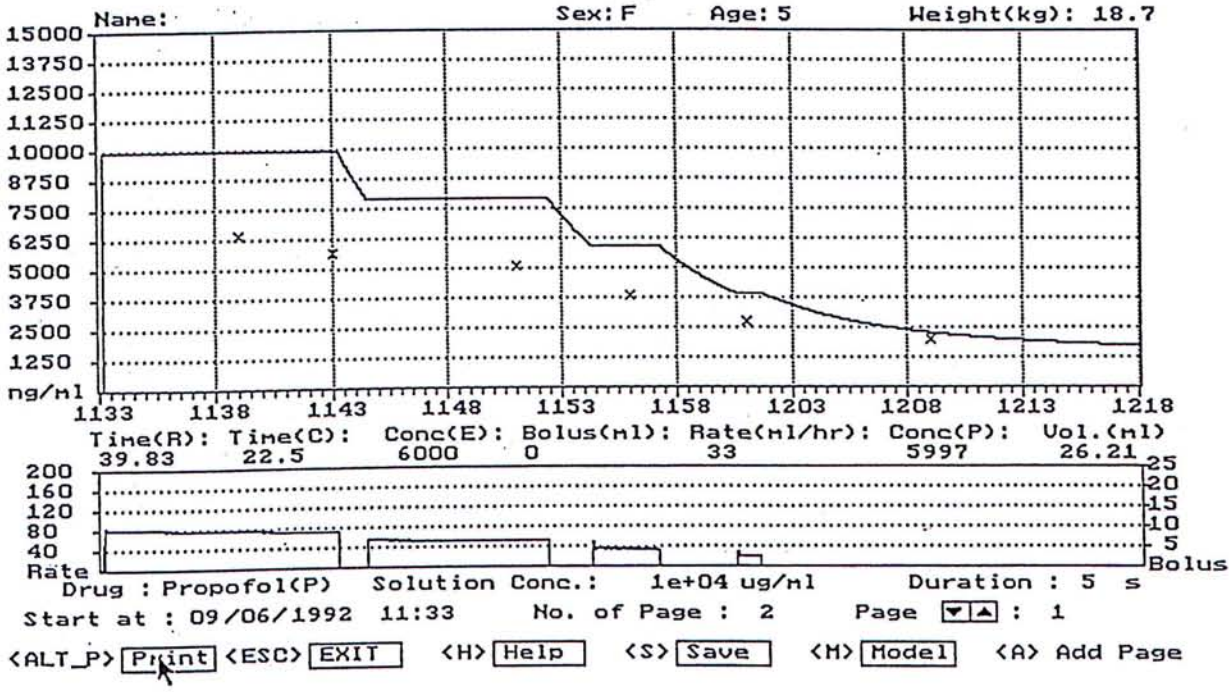
Drug information.....

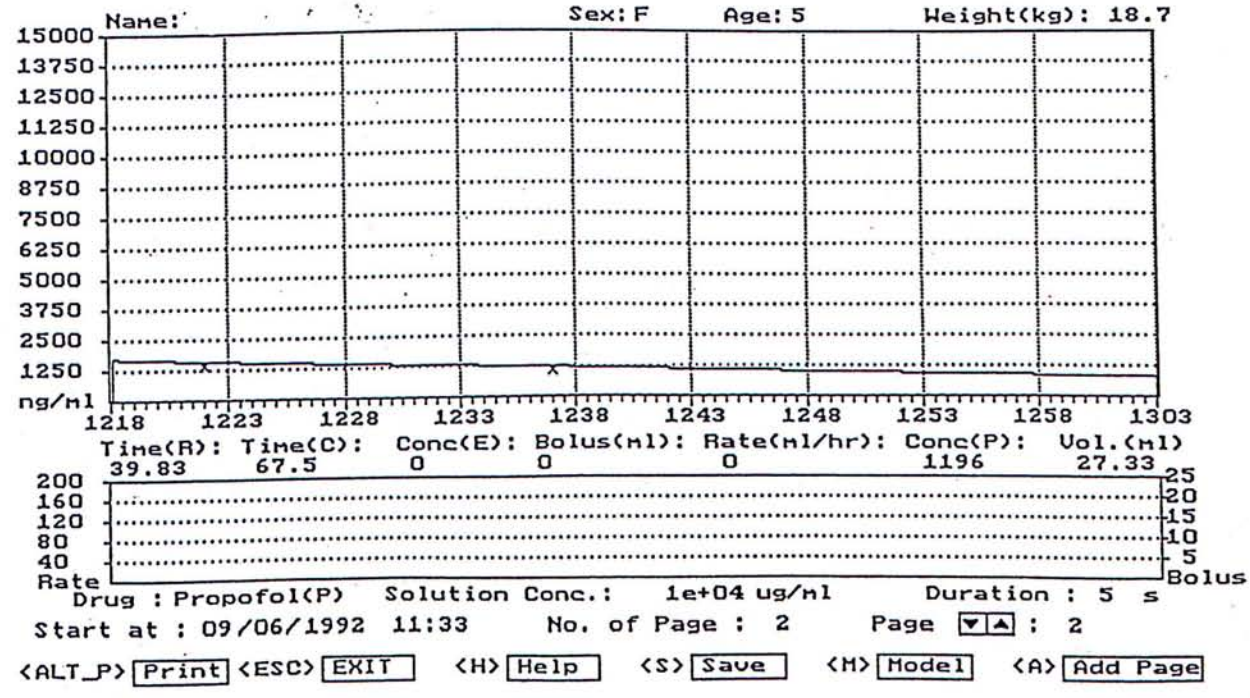
Volume ratio = 0.343000 (L/Kg) V1 = 6.414100 (L)

k10 = 0.100000 (/min) k12 = 0.085500 (/mi) k21 = 0.033000 (/min)

k13 = 0.021000 (/min) k31 = 0.003300 (/min)

Remark :





2.3: Comparison of measured and predicted blood concentrations of propofol

Throughout the study, measured blood concentrations of propofol (Cp) were compared with the corresponding delivery system predicted value. The prediction error was calculated for each data point, this being defined as (Glass *et al*, 1989).

$$\text{Prediction error} = \frac{\text{Cp (measured)} - \text{Cp (predicted)}}{\text{Cp (predicted)}} \times 100$$

Bias was defined as the mean prediction error and is taken in these circumstances to be a measure of the systematic tendency of the system to under or over-estimate the measured concentration of blood propofol. That is to say, if bias has a positive value, then the measured value is , on average, greater than the system prediction and *vice versa*. Precision is defined as the mean value of the sum of individual absolute values of prediction error and is a measure of the degree of scatter of the data about the line of perfect prediction. A two stage approach was used, because of the different numbers of blood samples taken from each patient. Bias and precision were calculated for each patient and then the population bias & precision calculated.

Precision (average absolute error) and bias (average error) ware calculated for each individual data set using the following equations:

$$\text{precision} = \frac{\sum_{j=1}^{n} \frac{|\hat{y}_j - y_j|}{y_j}}{n} \qquad \text{bias} = \frac{\sum_{j=1}^{n} \frac{\hat{y}_j - y_j}{y_j}}{n}$$

Where

n = number of measured propofol levels for each patient.

y = measured blood propofol concentration.

$\hat{y}$ = predicted blood concentration.

j = that population figures were derived.

Table 5-1:

The result of propofol infusion for Chinese children (first group)

	Vc (litre/kg)	K10 (/min)	K12 (/min)	K13 (/min)	K21 (/min)	K31 (/min)
Marsh's rate constants	0.3430	0.1000	0.0855	0.0210	0.0330	0.0033
Revised rate constants	0.4318	0.0967	0.1413	0.0392	0.1092	0.0049

Patient's Name	Sex	Age (years)	Weight (kg)	Drug (mg)	Marsh's rate constants			Revised rate constants		
					Error	Absolute error	Square error	Error	Absolute error	Square error
Pinfu-1	M	4	17.1	305	-17.54	17.54	3699037	8.21	8.21	637957
Pinfu-2	M	4	18.7	521	-28.22	29.53	4820600	-12.43	21.21	1278584
Pinfu-3	M	6	16.0	469	-2.35	6.51	947147	18.48	18.48	2647994
Pinfu-4	M	6	21.8	563	6.07	24.35	6137853	26.50	31.79	2849323
Pinfu-5	M	7	19.1	343	-38.22	38.22	8503323	-27.99	27.99	1850877
Pinfu-6	F	5	16.8	394	-30.11	30.17	9549856	-12.61	15.60	1786287
Pinfu-7	F	5	17.3	263	-8.03	12.73	1843105	17.00	24.94	442792
Pinfu-8	M	4	15.2	271	-41.17	41.17	11720586	-28.93	28.93	3171378
Pinfu-9	M	6	21.5	316	10.43	12.72	372220	28.58	31.48	3535899
Pinfu-10	F	5	18.7	273	-29.63	29.63	6003091	-17.45	19.46	1224472
Precision					24.26			22.81		
Bias					-17.88			-0.06		
MSE					5359682			1942556		

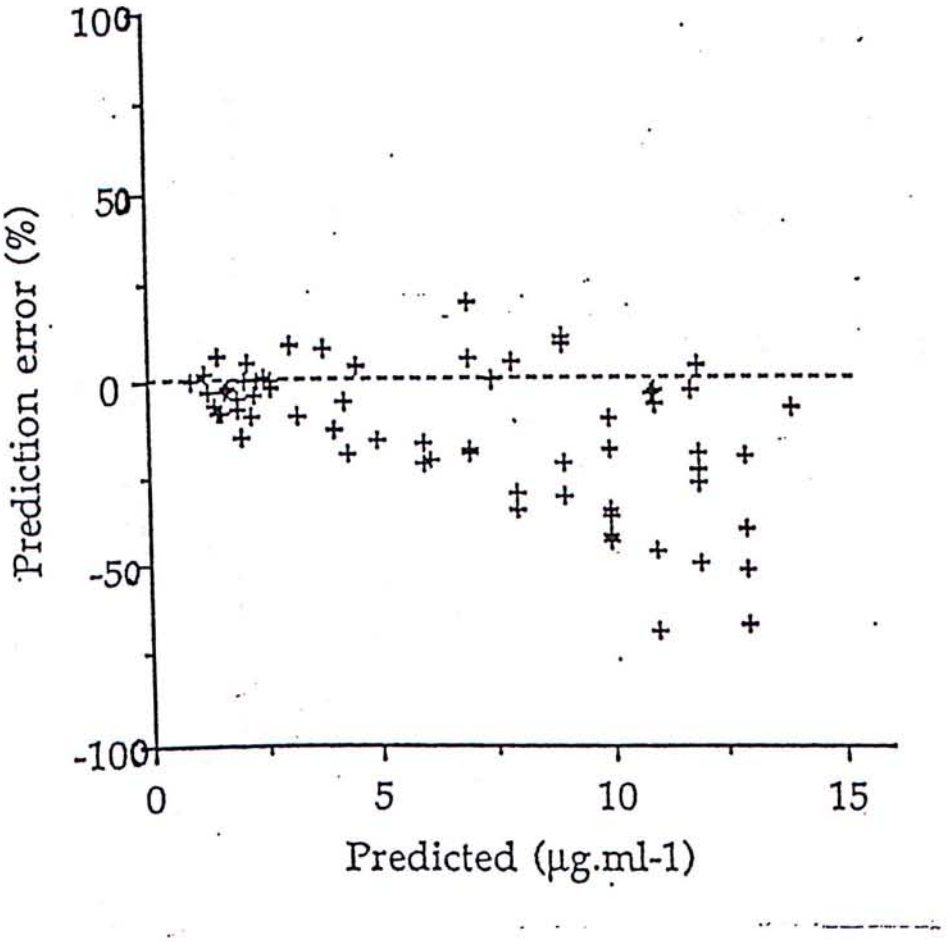


Fig. 5-3: Comparison of percentage prediction error and predicted concentration. n=67 (Based on Short *et al.* 1994).

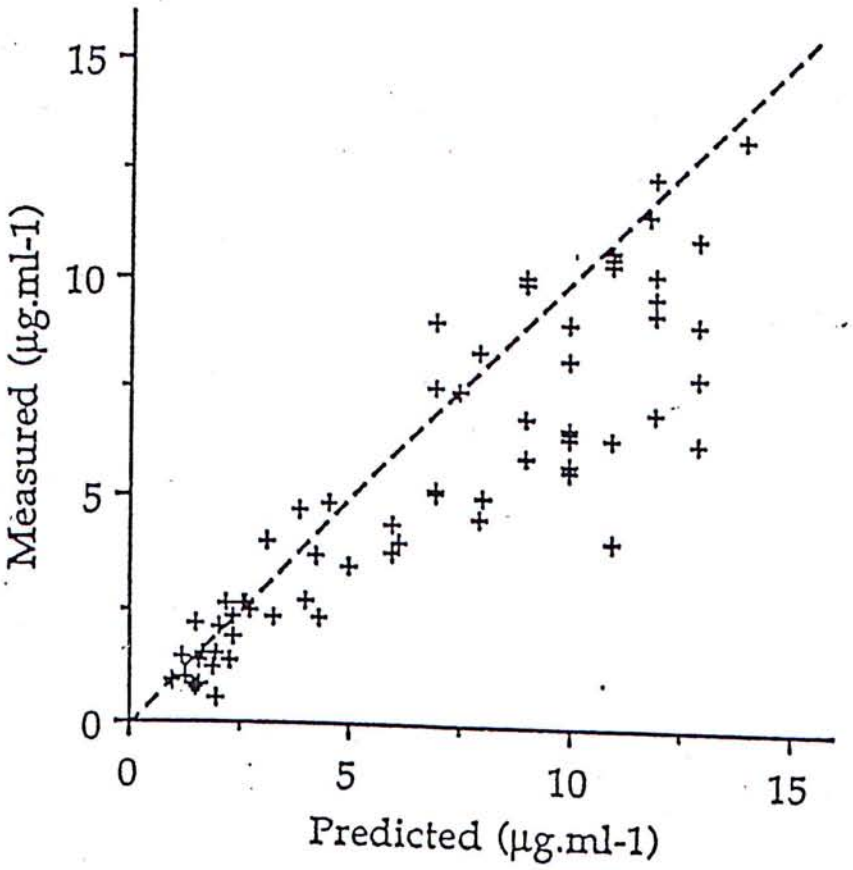


Fig. 5-4: Comparison of measured and predicted blood concentrations of propofol for the first study population (n=67, r=0.89). The dashed line is the line of identity (y=x) (Based on Short *et al.* 1994)

Table 5-2: Comparison between the Marsh's propofol infusion model and revised rate constants for propofol using a three compartment open model, and comparison of bias and precision (95% confidence limits). V_c=volume of the central compartment.

	Marsh's rate constants	Revised rate constants	
		1st Group	2nd Group
V_c (ml/kg)	343	432	432
k_{10} (/min)	0.1000	0.0967	0.0967
k_{12} (/min)	0.0855	0.1413	0.1413
k_{13} (/min)	0.0210	0.0392	0.0392
k_{21} (/min)	0.0330	0.1092	0.1092
k_{31} (/min)	0.0033	0.0049	0.0049
Bias (%)	-17.88 (-41 to 10)	-0.06 (-28 to 29)	0.19 (-30 to 42)
Precision (%)	24.26 (7 to 41)	22.81 (8 to 32)	21.06 (8 to 43)

1st Group: **Table 5-1:** The result of propofol infusion for Chinese children (first group)

2nd Group: **Table 5-3:** The result of propofol infusion for Chinese children (second group) detail in follow **2.4:** Test the new paediatric pharmacokinetic model.

Blood concentration for satisfactory anaesthesia ranged from 5-13 μg/ml. Mean total dose of propofol given was 372 mg (range 263-563 mg) and the mean rate of infusion was 29.8 mg/kg/h. The population mean prediction error (bias) was calculated to be -17.88% and the corresponding value for precision obtained was 24.26%. After linear least squares regression the revised rate constants had a precision of 22.81% and bias of -0.06% (**Table 5-2:** Revised rate constants: V_c = 432 ml/kg, k_{10} = 0.0967/min, k_{12} = 0.1413/min, k_{13} = 0.0392/min, k_{21} = 0.1092/min, k_{31} = 0.0049/min). Fit of the model was significantly improved ($p < 0.001$). Using a paired *t* test to combine each individual mean for Bias & Precision. (Short *et at.* 1994)

In the first study, each measured blood value of propofol was compared with its corresponding delivery system prediction and the results obtained were displayed in **Table 5-1:** The results of propofol infusion for Chinese children (first group). These results were plotted in the form of prediction error *vs* predicted concentration (**Fig 5-3:** Comparison of percentage prediction error and predicted concentration. n = 67). It may be seen from this figure that the majority of the data points lie below the line of zero prediction error (the line of perfect prediction).

2.4: Test the new paediatric pharmacokinetic model (the revised paediatric rate constants).

In order to test the new paediatric pharmacokinetic model prospectively, a second study was performed in the patients in second group [**Table 5-3:** The result of propofol infusion for Chinese children (second group)] In this second study, the computer controlled propofol infusion system was programmed with the revised paediatric rate constants. As before, measured blood concentrations of propofol were compared with their corresponding predicted values calculated by the delivery system algorithm. **Fig 5-5:** (Comparison of measured and predicted blood concentrations of propofol for the second study population) shows the relationship between measured and predicted values for the entire prospective study population and **Fig 5-6:** (Comparison of percentage prediction error and new predicted value using the newly derived paediatric pharmacokinetic parameters for propofol n = 172) shows the distribution of prediction errors in relation to their predicted values. The bias of the revised rate constants delivery system was calculated to be 0.19% and the corresponding value of precision was 21.06%. It can be seen that, prospectively, the revised paediatric rate constant system gave a relationship between measured and predicted values of blood propofol which was superior to that obtained using the rate constants system based on the

paediatric data estimated by Marsh & colleagues used in the initial study. It also compared closely to the relationship predicted by computer simulation using the revised paediatric rate constant system.

Table 5-3

The result of Propofol infusion for Chinese children (second group)

		Vc	K10	K12	K13	K21	K31
		(litre/kg)	(/min)	(/min)	(/min)	(/min)	(/min)
Revised rate constants		0.4320	0.0967	0.1413	0.0392	0.1092	0.0049

Patient's Name	Sex	Age	weight (kg)	Drug (mg)	Revised rate constants		
					Error	Absolute error	Square error
Pinfu-11	M	4	16.7	270	2.27	16.56	1505740
Pinfu-12	M	6	20.3	408	-10.96	10.96	326121
Pinfu-13	F	9	33.0	736	7.89	14.66	1014033
Pinfu-14	M	10	25.0	797	8.26	13.99	1233897
Pinfu-15	M	8	18.8	344	24.86	24.86	602237
Pinfu-16	M	5	18.8	537	-13.74	18.16	1892876
Pinfu-17	M	6	17.1	321	-5.29	12.34	387495
Pinfu-18	M	6	19.4	376	23.36	24.23	3488355
Pinfu-19	M	6	24.5	271	-17.20	20.28	449052
Pinfu-20	M	4	16.5	222	6.23	16.01	1268514
Pinfu-21	M	9	29.7	419	-0.35	16.93	869385
Pinfu-22	M	4	15.3	171	-19.56	23.56	904747
Pinfu-23	M	9	23.3	410	-17.73	18.52	828588
Pinfu-24	M	10	27.0	377	-21.30	34.27	2590242
Pinfu-25	M	9	43.7	797	42.44	43.09	3393649
Pinfu-26	M	4	16.3	302	-30.47	30.47	3321115
Pinfu-27	M	5	19.6	299	1.54	8.41	320370
Pinfu-28	M	6	23.6	314	15.08	22.67	2322222
Pinfu-29	M	9	39.0	623	22.44	30.84	3428072
Pinfu-30	M	8	26.7	317	-13.96	20.41	421403
Precision					21.06		
Bias					0.19		
MSE					1528406		

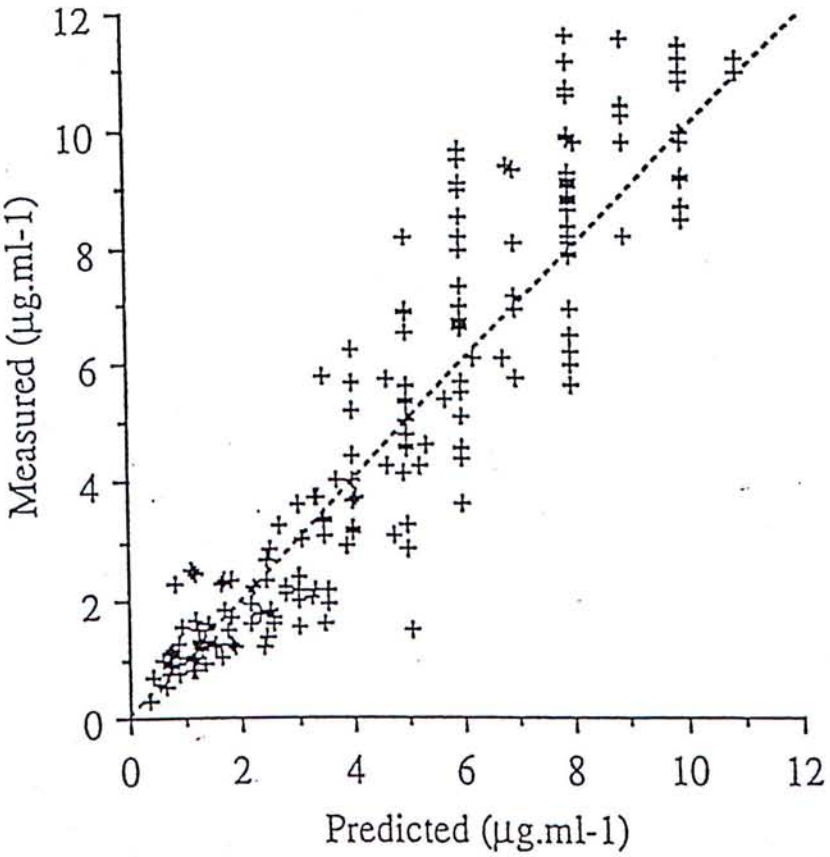


Fig. 5-5: Comparison of measured and predicted blood concentrations of propofol for the second study population. The predicted values were derived by weighted least squares regression using the derived paediatric pharmacokinetic parameters for propofol (n=172). Dotted line represents the line of identity (y=x) (Based on Short *et al*, 1994).

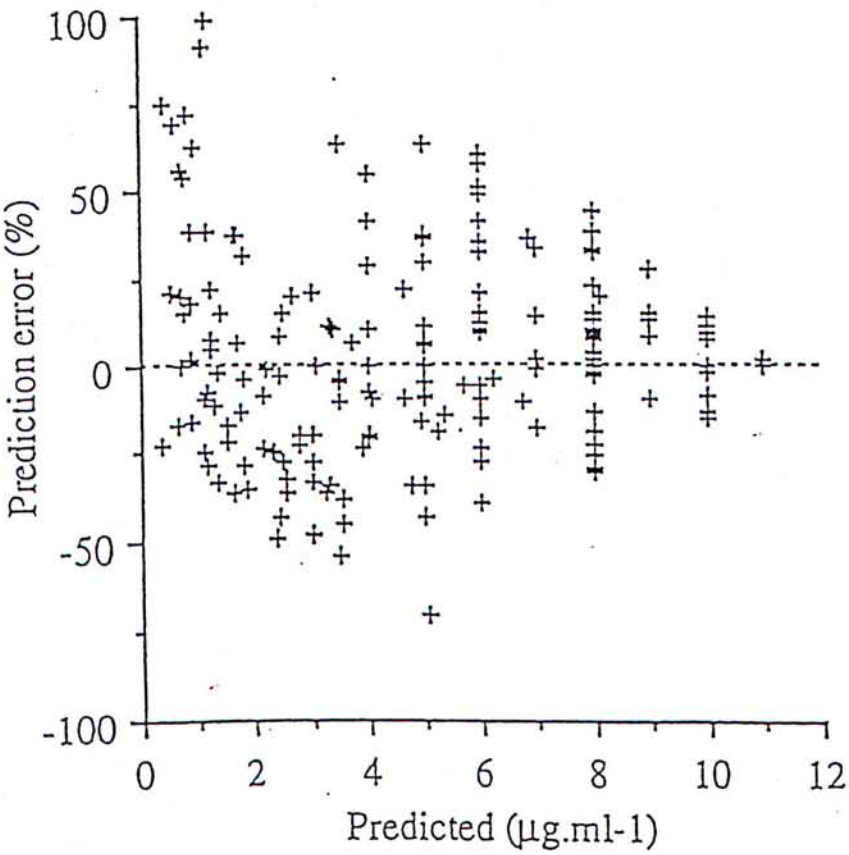


Fig. 5-6: Comparison of percentage prediction error and new predicted value obtained by pharmacokinetic simulation using the derived paediatric pharmacokinetic parameters for propofol (n=172) (Based on Short et al, 1994).

3: Discussion of propofol pharmacokinetics studies: infusion for Chinese children

In this study we have evaluated prospectively a pharmacokinetic model-driven infusion of propofol based on that of Marsh and colleagues (Marsh *et al.* 1991). This model had been developed using children of similar age and undergoing similar procedures to the patients in this study. Its accuracy had been confirmed by prospective testing. When compared with their data, in our patients the precision model was lower (24.26% *vs* 22.81%, 21.06%) and there was a large negative bias: the algorithm underestimated blood concentrations by 17.88%. (described as **Table 5-2:** Comparison between the Marsh's propofol infusion model and revised rate constants for propofol using a three compartment open model, and comparison of bias and precision). This indicated that the pharmacokinetic data of Marsh and colleagues were not applicable to our patients. Therefore, we revised the pharmacokinetic variables and tested the new variables prospectively, when they were found to fit our population well. This confirms the difference in the pharmacokinetic profiles of the children in the two studies (Short *et al.* 1994).

The reasons for the difference in pharmacokinetic variables between the present study and that of Marsh and colleagues (Marsh *et al.* 1991) are unclear. The main

differences between the two studies were that our study was of Chinese children and our anaesthetic technique did not include sedative premedication. Analgesia in the form of a local anaesthetic block was also performed at the end of surgery rather than at the beginning, in the first phase of the study. However, although the change to performing the local anaesthetic block before surgery for the second part of the study reduced propofol requirements, it did not alter significantly the pharmacokinetic profile of propofol. The volume of distribution of the central compartment in our model was 25% larger than that of Marsh and colleagues and is close to the value obtained in a previous pharmacokinetic study of a bolus dose of propofol in Chinese children (Jones et al. 1990). However, caution must be exercised in interpreting the pharmacokinetic variables in the model after it has been subjected to an iterative least squares regression procedure, because the relative contribution of each variable to the overall model is influenced by its order in the regression procedure. The revised model is regarded best as a mathematical algorithm that best fits the blood concentration-time profile of the drug, rather than a true compartmental pharmacokinetic model (Short *et al.* 1994).

CHAPTER SIX

CLINICAL APPLICATION

ON PROTEIN BINDING STUDIES

1: Plasma proteins and drug binding

The pharmacological response obtained is, for the majority of drugs, related to the concentration of drug at its receptor site(s). The disposition and ultimate biological effect of the drug may be said to be 'influenced' by the extent of plasma protein binding. Any significant alteration in plasma protein binding can lead to an altered drug distribution and unexpected pharmacological responses. A change in protein binding also has implications for therapeutic drug monitoring.

Human plasma contains over 60 different proteins. Plasma albumin is the most abundant protein in plasma, accounting for approximately 50% of total proteins. It exerts 80% of the colloid osmotic pressure of blood and is therefore an essential factor in the maintenance of blood volume. The interaction between proteins and drugs is

governed by the law of mass action. Plasma protein binding of drugs is usually reversible (the half-life $t_{1/2}$ being in the order of milliseconds) and, generally, changes in protein binding only cause significant clinical effects for drug that are highly protein bound because it is the free drug that exerts the effect. A 1% decrease in binding capacity for a drug that is normally 99% bound to plasma proteins would have the effect of doubling the 'free' drug available for distribution and metabolism. The principal plasma proteins involved in drug binding are albumin, glycoproteins and lipoproteins.

There are changes in protein position in children and also in pregnancy and several studies were performed to determine whether there were any changes in protein binding in these population.

2: Methods of propofol protein binding studies

2.1: Blood sample acquisition

The studies was approved by the Clinical Research Ethical Committee and informed consent was obtained from all patients or volunteers. Patients selected were healthy (ASA I or II). Venous blood was collected into a heparinized tube and sent immediately to the laboratory for propofol protein binding study (level 2000-3000 ng/ml).

2.2: Population characteristics

Venous blood was taken from 6 groups of subjects.

A. Non pregnant, pre-menopausal women, who were not taking hormonal contraceptives.
B. Patients scheduled for elective Caesarean section at 38-42 weeks gestation.
C. Umbilical venous blood obtained from the placenta after elective Caesarean section at 38-42 weeks gestation.
D. Children between 1-3 years.
E. Children between 3-12 years.
F. Adults 18-40 years.

2.3: Methods of protein binding assay

Refer to **Chapter Four: 2: Methods of protein binding of propofol** for details on protein binding assay.

3: Results of propofol protein binding

The results of propofol protein binding described in **Table 6-1:** The result of propofol protein-binding study (Children & Adult), **Table 6-3:** Population characteristics in propofol protein-binding (Children & Adult), and **Table 6-2:** The result of propofol protein-binding study (Pregnant & Neonate), **Table 6-4:** Population characteristics in propofol protein-binding (Pregnant & Neonate).

Table 6-1:

The result of propofol protein-binding study

(Children & Adult)

No	Patient Name	Sex	Age	Weight (kg)	Group	Buffer		Plasma		Total	Bound
						Ratio	ng/mL	Ratio	ng/mL	(ng/mL)	(%)
1	Pprobin-1	M	1	11.80	<3yr	0.0574	27.78	4.6966	2273.15	2300.94	**98.78**
2	Pprobin-2	M	1	10.90		0.0225	11.66	2.8509	1476.77	1488.42	**99.21**
3	Pprobin-3	M	1	11.80		0.0456	22.07	4.5859	2219.58	2241.65	**99.01**
4	Pprobin-4	M	2	11.70		0.0407	22.34	3.0802	1691.03	1713.37	**98.68**
5	Pprobin-5	M	2	12.50		0.0981	57.19	5.9229	3453.05	3510.24	**98.34**
6	Pprobin-6	M	2	12.40		0.0207	12.07	2.1787	1270.18	1282.25	**99.05**
7	Pprobin-7	F	2	9.90		0.0567	27.44	3.8940	1884.70	1912.14	**98.54**
8	Pprobin-8	M	2	13.90		0.0711	34.41	4.0293	1950.18	1984.59	**98.24**
9	Pprobin-9	M	3	14.60		0.0355	19.49	3.9657	2177.17	2196.66	**99.10**
10	Pprobin-10	M	3	14.50		0.0320	18.66	2.7794	1620.39	1639.05	**98.85**
11	Pprobin-11	M	3	13.40		0.0432	23.72	3.2974	1810.27	1833.99	**98.69**
12	Pprobin-12		3	16.70		0.0491	23.76	3.7902	1834.46	1858.22	**98.70**
13	Pprobin-13	M	4	12.90	3-12y	0.0462	22.36	3.9368	1905.41	1927.77	**98.83**
14	Pprobin-14	M	4	15.70		0.0382	18.49	3.9980	1935.03	1953.52	**99.04**
15	Pprobin-15	M	8	27.00		0.0353	20.58	3.1305	1825.08	1845.66	**98.87**
16	Pprobin-16	M	10	51.00		0.1232	67.64	6.5717	3607.86	3675.50	**98.13**
17	Pprobin-17	F	10	31.20		0.0311	17.07	3.1399	1723.81	1740.88	**99.01**
18	Pprobin-18	M	11	39.50		0.0351	19.27	3.3469	1837.45	1856.72	**98.95**
19	Pprobin-19	M	11	36.00		0.11	60.39	5.9234	3251.95	3312.34	**98.14**
20	Pprobin-20	M	12	30.00		0.0533	25.80	3.7754	1827.29	1853.09	**98.59**
21	Pprobin-21	M	12	38.00		0.0545	29.92	4.0748	2237.07	2266.99	**98.66**

Table 6-1:

The result of propofol protein-binding study

(Children & Adult)

No	Patient Name	Sex	Age	Weight (kg)	Group	Buffer		Plasma		Total	Bound
						Ratio	ng/mL	Ratio	ng/mL	(ng/mL)	(%)
22	Pprobin-22	M	21	64.00	Adult	0.0597	33.49	3.1008	1739.11	1772.61	98.07
23	Pprobin-23	M	25	72.00		0.0517	26.78	3.0663	1588.34	1615.12	98.31
24	Pprobin-24	M	26	64.00		0.0430	25.07	2.6832	1564.31	1589.37	98.40
25	Pprobin-25	M	27			0.0582	32.65	3.3006	1851.15	1883.81	98.24
26	Pprobin-26	M	28	70.00		0.0461	23.88	2.8374	1469.77	1493.65	98.38
27	Pprobin-27	M	31	66.00		0.0360	20.99	3.0618	1785.03	1806.02	98.82
28	Pprobin-28	M	32	64.00		0.0467	24.19	2.8719	1487.64	1511.83	98.37
29	Pprobin-29	M	37	62.00		0.0446	26.00	2.6700	1556.61	1582.61	98.33
30	Pprobin-30	M	30	77.00		0.0345	19.34	2.8999	1626.39	1645.73	98.81
31	Pprobin-31	M	34	66.00		0.0719	40.32	3.2351	1814.39	1854.71	97.78

Table 6-2:

The result of propofol protein-binding study

(Pregnant & Neonate)

No	Patient Name	Sex	Age	Weight (kg)	Group	Buffer		Plasma		Total (ng/mL)	Bound (%)
						Ratio	ng/mL	Ratio	ng/mL		
32	Pprobin-32	F			Pregnant	0.1029	49.80	5.9381	2874.04	2923.84	98.27
33						0.0991	47.96	5.7521	2784.02	2831.98	98.28
34	Pprobin-33	F				0.0929	44.96	6.6256	3206.79	3251.75	98.60
35						0.1040	50.34	6.6491	3218.16	3268.50	98.44
36	Pprobin-34	F				0.0669	32.38	6.0775	2941.51	2973.89	98.90
37						0.0584	28.27	5.2134	2523.29	2551.55	98.88
38	Pprobin-35	F				0.0748	36.20	6.2730	3036.13	3072.34	98.81
39						0.0752	36.40	6.1608	2981.83	3018.22	98.78
40	Pprobin-36	F				0.0801	38.77	5.4511	2638.33	2677.10	98.53
41						0.0762	36.88	6.5160	3153.74	3190.62	98.83
42	Pprobin-37	F				0.0521	25.22	3.1912	1544.54	1569.76	98.37
43						0.0563	27.25	3.4178	1654.22	1681.46	98.35
44	Pprobin-38	F				0.0718	37.19	4.7278	2449.00	2486.19	98.48
45						0.0461	23.88	4.1592	2154.47	2178.35	98.89
46	Pprobin-39	F				0.1006	48.69	6.7624	3273.00	3321.69	98.51
47	Pprobin-40	F				0.0573	29.68	5.1213	2652.83	2682.51	98.88
48	Pprobin-41	F				0.0626	32.43	3.0017	1554.88	1587.31	97.91
49	Pprobin-42	F				0.0513	26.57	4.7104	2439.99	2466.56	98.91
50	Pprobin-43	F				0.0723	39.69	5.5340	3038.17	3077.86	98.69
51	Pprobin-44				Neonate	0.1173	56.77	3.9544	1913.93	1970.70	97.03
52						0.1282	62.05	3.6494	1766.31	1828.36	96.49
53	Pprobin-45					0.1363	65.97	4.0764	1972.98	2038.95	96.66
54						0.1433	69.36	3.9749	1923.85	1993.21	96.39
55	Pprobin-46					0.1097	53.09	3.3119	1602.96	1656.05	96.69
56						0.1041	50.38	3.3501	1621.45	1671.83	96.89
57	Pprobin-47					0.1138	55.08	3.4199	1655.23	1710.31	96.67

Table 6-2:

The result of propofol protein-binding study

(Pregnant & Neonate)

No	Patient Name	Sex	Age	Weight (kg)	Group	Buffer		Plasma		Total	Bound
						Ratio	ng/mL	Ratio	ng/mL	(ng/mL)	(%)
58						0.1186	57.40	3.5085	1698.11	1755.52	96.62
59	Pprobin-48					0.1254	60.69	3.2772	1586.16	1646.86	96.17
60	Pprobin-49					0.1180	57.11	5.1971	2515.40	2572.51	97.73
61	Pprobin-50					0.1468	76.04	3.4647	1794.71	1870.76	95.76
62						0.1316	68.17	3.3970	1759.65	1827.81	96.13
63	Pprobin-51					0.1484	71.83	4.4314	2144.80	2216.62	96.65
64	Pprobin-52					0.1047	54.23	2.8112	1456.20	1510.44	96.28
65	Pprobin-53					0.0954	49.42	2.9241	1514.68	1564.10	96.74
66	Pprobin-54					0.1066	55.22	2.3627	1223.88	1279.10	95.49
67	Pprobin-55					0.0943	51.77	3.7030	2032.95	2084.72	97.45
68	Pprobin-56	F			Non-	0.0556	26.91	5.9442	2876.99	2903.90	99.06
69	Pprobin-57	F			Pregnant	0.0384	18.59	3.9189	1896.75	1915.33	99.02
70	Pprobin-58	F				0.0908	43.95	5.9576	2883.48	2927.43	98.48
71	Pprobin-59	F				0.0561	27.15	5.8884	2849.99	2877.14	99.05
72	Pprobin-60	F				0.0228	11.04	2.7094	1311.35	1322.38	99.16
73	Pprobin-61	F				0.0180	8.71	3.4247	1657.55	1666.27	99.47
74	Pprobin-62	F				0.0891	43.12	6.3102	3054.14	3097.26	98.59
75	Pprobin-63	F				0.0961	46.51	7.2846	3525.75	3572.26	98.68
76	Pprobin-64	F				0.0490	23.72	4.7672	2307.32	2331.04	98.97
77	Pprobin-65	F				0.0306	17.84	3.6956	2154.53	2172.37	99.17
78	Pprobin-66	F				0.0201	9.73	2.4116	1167.21	1176.94	99.17
79	Pprobin-67	F				0.0838	46.01	6.6488	3650.19	3696.20	98.74
80	Pprobin-68	F				0.0458	25.14	4.1952	2303.16	2328.31	98.91
81	Pprobin-69	F				0.0337	16.31	2.6602	1287.54	1303.85	98.73
82	Pprobin-70	F				0.0775	37.51	6.3739	3084.97	3122.48	98.78
83	Pprobin-71	F				0.0634	34.81	5.2020	2855.90	2890.70	98.78

Table 6-3:

Population characteristics in propofol protein-binding

(Children & Adult)

Groups of patients	Age (years)				Body weight (kg)				Protein-Binding (%)			
	n	Mean	S.D.	C.V.(%)	n	Mean	S.D.	C.V.(%)	n	Mean	S.D.	C.V.(%)
Young children	12	2.09	0.73	34.72	12	12.84	1.79	13.92	12	98.77	0.29	0.29
Children	9	8.08	3.91	48.41	9	31.26	11.18	35.76	9	98.69	0.33	0.33
Adult	10	29.10	4.44	15.25	10	67.22	4.57	6.79	10	98.35	0.29	0.30

Table 6-4:

Population Characteristics

in propofol protein-binding

(pregnant & Neonate)

Groups of patients	n	Protein-Binding (%) Mean	S.D.	C.V.(%)
Pregnant	19	98.60	0.27	0.28
Neonate	17	96.58	0.53	0.55
Non-Pregnant	16	98.92	0.25	0.25

4: Discussion of propofol protein binding

The binding of drugs to proteins, especially plasma proteins, can have a marked influence on their pharmacokinetics and pharmacodynamics and, therefore, on therapeutic drug monitoring. Nonprotein bound drug is the portion best correlated with pharmacologic activity and the pharmacokinetic characteristics of the total drug. Plasma and tissue protein binding is intimately involved in determining a particular drug's volume of distribution, transport to and from the site of action or degradation, and elimination from the body. Disease states, concomitant drug therapy, genetic, and age-related factors can alter the extent and affinity of protein binding and thus affect the pharmacokinetic important free drug concentrations. Propofol analysis normally measures total (free + bound) drug, and any alteration in the free:bound drug ratio may have implications in calculation of the dose. Therefore, a knowledge of the conditions under which changes in the plasma protein binding of drugs occur, and the extent of those changes, is valuable in predicting response and possible dose adjustment.

4.1: Protein binding of propofol in Chinese children

Children have been shown to require a larger dose of propofol than adults (Aun *et al*, 1992). Propofol is highly protein bound (97% - 99%), in young children (< 3 years old), we find the value has a highly protein-binding for propofol [98.77 ± 0.29 % (mean ± S.D.)], and the older children (3-12 years old) has also highly protein-binding [98.69 ± 0.33 % (mean ± S.D.)] [described in **Table 6-3:** Population characteristics in propofol protein-binding (Children & Adult)]. Compared the young children & children with adult [98.35 ± 0.29 % (mean ± S.D.)] [described in **Table 6-3:** Population characteristics in propofol protein-binding (Children & Adult)]. It show no significant difference in the propofol protein-binding between children and adult, using an unpaired *t* test. The propofol protein binding analysis study therefore suggests that the difference in the dose requirement between children and adults was not due to the differences in protein binding (unpublished data from Aun *et al*, 1994).

4.2: Protein binding of propofol in Pregnant women & neonate

Changes in the composition of maternal plasma/serum during normal pregnancy are now well documented. As well as a quantitative change in the normal proteins, there occurs during gestation synthesis of plasma proteins which are not normally detected in non-pregnant women. In addition there are changes in plasma concentrations of free fatty acids, hormones and other endogenous ligands, as well as large changes in body composition that may affect drug binding and ultimately response. There is an overall fall in the level of total plasma proteins during pregnancy, approximately 7% decrease at 6 months' gestation and some 12% at term (Haram *et al.* 1983). The degree of change recorded varies between investigators, in that some report a continuous reduction of total protein during pregnancy (Reboud *et al.* 1963). The level of albumin falls during gestation, as a result of the increase in plasma volume. In pregnancy, total body water increases by up to 8 L, of which 80% is extrcellular water. Plasma volume increases by 40 to 50%. This increase in plasma volume represents 1.2 to 1.5 L, adding considerably to the normal plasma volume of 2.5 L.

Neonatal drug disposition varies from that in adult and older infants as a result of differences in body composition, metabolism, kidney function, etc. Changes also

occur in plasma composition which affect the plasma protein binding of drugs and the fraction of free drug. At birth a full-term infant has a significantly lower plasma albumin level than adults, and therefore correspondingly fewer drug binding sites. Since the amount of propofol bound is related to the total albumin, there may be altered disposition in the neonate. The major protein detected in the newborn during the first 3 weeks of life is α-Feto-protein which has a lower affinity for drugs than 'adult' albumin (Miyoshi *et al.* 1966). As the fetal protein disappears from the circulation it is 'replaced' by albumin. The rapid decline of this protein, with its altered binding, may explain the rapidly changing pharmacokinetics of some drugs over the first 2 to 3 weeks of life.

Pregnant patients had increased free propofol, (1.41 ± 0.30%, mean ± S.D.) compared with nonpregnant patients, (1.08 ± 0.26%, $p = 0.004$). Although these findings would imply that the total propofol concentration of propofol, and hence the dose, could be reduced in pregnant patients for a given effect, changes in pharmacokinetics during pregnancy (Gin *et al*, 1991) and the increased total body weight in pregnancy could also affect dose requirements. These results indicate that further simultaneous pharmacodynamic/pharmacokinetic modelling would be useful to determine the clinical significance of the changes in protein binding.

Free propofol in umbilical plasma (3.40 ± 0.61%, mean ± S.D.) was greater than that in maternal (pregnant) plasma (1.41 ± 0.30%, p = 0.001). These results are consistent with the umbilical: maternal ratio of propofol found at Caesarean section (Gin *et al*, 1991). However propofol is bound to red blood cell as well as albumin. The different composition of umbilical blood compared with maternal blood could also influence the protein binding and free fraction of propofol. The clinical significance of free propofol, compared with other anaesthetic agents, has not been evaluated. However, propofol concentrations in umbilical blood at Caesarean section are relatively low, neonatal outcome is not worse that that after other induction agents, and neonatal elimination of propofol is probably faster that that of thiopentone (Gin *et al*, 1991). At present, the manufacturers of propofol do not recommend its use in obstetric or neonatal anaesthesia. Thus it is not likely that further studies will be carried out at this time.

CHAPTER SEVEN

CONCLUSIONS

Propofol is a commercially available intravenous anaesthetic agent. The main feature of propofol is rapid recovery from anaesthesia whether given as a bolus for induction of anaesthesia or by infusion to maintain anaesthesia.

I have been able to develop two analytic methods for the assay of propofol: high pressure liquid chromatography analysis for propofol quantitation and equilibrium dialysis for separation of propofol in whole blood into free and protein bound fractions. The methods permit the determination of propofol in whole blood and plasma over a wide range of concentrations.

High pressure liquid chromatography has become the most popular chromatographic method, because HPLC allows small samples to be used, especially with low dead volume detectors coupled with strip chart recorder. The combination of HPLC and fluorescence detection has improved the sensitivity of propofol measurements. The method

described in this thesis is more rapid and simpler to use than the indophenol procedure and has an improved limit of quantification of 2 ng/ml. The reversed phase HPLC method used was simple, sensitive, and reliable. The assay involved a single extraction of the drug and internal standard, thymol, from blood buffered with 0.1 M sodium dihydrogen phosphate and extracted into cyclohexane. The organic extract, basified with tetramethylammonium hydroxide, was evaporated to dryness at 37°C under nitrogen. The residue was redissolved in 80 μl acetonitrile and an aliquot of the concentrate was injected into a C18 reversed-phase column. The mobile phase consisted of 66% (v/v) acetonitrile in Milli-Q water containing 1% v/v acetic acid and was eluted at 1.7 ml/min. The components of the column effluent was monitored by a fluorometric detector with excitation and emission wavelengths set at 276 nm and 310 nm, respectively. The method uses only 0.5 ml of sample and this facilitates pharmacokinetic studies of propofol in children (The method was based on that published by Chan & So 1990). The between batches coefficient of variance were 9.7850 % at 50 ng/ml and 7.6469 % at 3000 ng/ml. The intraassay coefficient of variation of propofol in whole blood was 6.24% at 100 ng/ml and 6.10% at 500 ng/ml, while the interassay coefficient of variation was 3.18% at 100 ng/ml and 1.81% at 500 ng/ml. The extraction recovery results obtained for propofol in whole blood are 104% at 100 ng /ml and 99% at 500 ng/ml.

Precision & accuracy and extraction efficiency of analytical method are all acceptable. A slight decrease in the amount of propofol was detected after 30 days. The results of propofol assay in plasma are similar to propofol assay in whole blood.

Equilibrium dialysis was performed using a Spectrum equilibrium Dialyser. Drug-containing plasma samples or protein solutions (1 ml) were dialysed against drug-free Sorensen's phosphate buffer (1 ml; pH 7.4) in teflon dialysis chambers separated by Spectra/Por dialysis membrane. The teflon cells rotate at 15 rpm at 37°C. The optimum dialysis time was 240 min. Propofol concentration in samples were assayed by the HPLC method described above. The intraassay coefficient of variation of total propofol in plasma was 1.97% at 3000 ng/ml, protein bound propofol was 0.11% and free propofol was 3.65%. The interassay coefficient of variation of total propofol in plasma was 2.19% at 3000 ng/ml, protein bound propofol was 0.23% and free propofol was 8.57%. The extraction recovery results obtained for propofol in plasma are 96.42% for unheated samples, 90.64% for samples heated at 37°C, and 97.15% for samples dialysised at 37°C in a water bath.

One application of my research was the prospective testing in chinese children of a previously published pharmacokinetic model driven algorithm for computer

controlled infusion of propofol. The concept of computer controlled infusion devices to deliver i.v. anaesthetic drugs according to pharmacokinetic model-driven algorithms is attractive. Drugs may be administered to a theoretical target blood concentration calculated by the algorithm. All relevant pharmacokinetic data are contained in the algorithm implying that administration of the infusion is simplified and there is no need to remember formulae for delivering the infusion by manual adjustment. Successful use of these algorithms requires a knowledge of blood concentration-effect relationships for the drug and of the accuracy of the algorithm. In this study we tested prospectively the use of an algorithm published previously for administering propofol to paediatric patients with precision of 24.26% and bias of -17.88%. The model was revised using an iterative linear least squares regression and minimizing total mean squared prediction error for each patient. The precision of the revised model was 21.08%, bias was 0.19%, and the fit of the model was significantly improved ($p < 0.001$).

Another application of my research was to investigate protein binding of propofol in different populations because pharmacological response to drugs is influenced by the free fraction of drug. Propofol protein binding was estimated by equilibrium dialysis, to be 97-99% bound in children and adults. From our studies, young children (< 3

years old) have protein-binding of 98.77 ± 0.29% (mean ± S.D.), children 3-12 years old have protein-binding of 98.69 ± 0.33%, and adult have protein-binding of 98.35 ± 0.29%.

Pregnant patients had increased free propofol, 1.41 ± 30% compared with nonpregnant patients, 1.08 ± 0.26% ($p = 0.004$). These findings indicate that further simultaneous pharmacodynamic/pharmacokinetic studies would be useful to determine the clinical significance of changes in protein binding. Free propofol in umbilical plasma 3.40 ± 0.61% was greater than that in maternal (pregnant) plasma ($p = 0.001$). The clinical significance of free propofol in neonates, compared with other anaesthetic agents, has not been evaluated. However, propofol concentrations in umbilical blood at Caesarean section are relatively low, neonatal outcome is not worse that that after other induction agents, and neonatal elimination of propofol is probably faster that that of thiopentone. At present, the manufacturers of propofol do not recommend its use in obstetric or neonatal anaesthesia. Thus it is not likely that further studies will be carried out at this time.

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APPENDIX

Acetonitrile

CH_3CN HPLC Grade, MERCK Art 2856, E. Merck, Frankfur, Germany.

Acetic acid

CH_3COOH glacial 100% Guarantee grade, MERCK Art 2500, E. Merck, Frankfur, Germany.

Centrifuge

BECKMAN CPR centrifuge, BECKMAN Instruments INC, Fullerton, California, U.S.A.

Column Nova-pak C18 ODS

Waters division of MILLIPORE, Milford, MA, U.S.A.

Cyclohexane

$CH_2.(CH_2)4.CH_2$=84.16 AnalaR UN 1145, BDH Limited, Poole Dorset, England.

Di-sodium hydrogen phosphate

$Na_2HPO_4.2H_2O$ 11.87 g/L M=137.99 g/mol guarantee grade, MERCK Art 6586, E. Merck, Frankfur, Germany.

Fluorescence spectrophotometer

650-40 model, HITACHI LTD, Tokyo, Japan.

Hydrochloric Acid

C(HCl)=1 mol/L (1N) Volumetric solution MERCK, Art 9057, E. Merck, Frankfur, Germany.

Injection system

RHEODYNE COTARI 7010 Modle, RHEODYNE INCORPORATED, Cotat, California, U.S.A.

Integrator

HP 3396A HEWLETT PACKARD Company, Atlanta, Georgia, U.S.A.

Metering pump

LDC/Milton Roy constaMetric*IIIG, Riviera Beach, Florida, U.S.A.

Methanol

CH_3OH, HPLC grade, MERCK Art 3041, E. Merck, Frankfur, Germany.

Mill-Q water

Tap water through the Mill-Q Reagent water system. MILLIPORE Corporation, Bedford, MA, U.S.A.

Mixture

THERMOLYNE Speci-Mix SYBRON Corporation, Dubuque, Iowa, U.S.A.

Molecularporous Dialysis Membrane

Spectra/Por, SPECTRUM MEDICAL IND., INC, Los Angeles, California, U.S.A.

Ohmeda 9000 syringe pump

Ohmeda, West Yorkshire, U.K

PH-Meter

CG 804 SCHOTT-GER'A'TE GmbH, Postfach, Hofheim a. Ts., Germany.

Potassium dihydrogen phosphate

KH_2PO_4 9.073 g/L M=136.09 g/mol Guarantee grade, MERCK Art 4873, E. Merck, Franfurt, Germany.

2-Propanol (IPA)

Guarantee grade, MERCK Art 9634, E. Merck, Franfurt, Germany.

Propofol stock solutions

ICI Pharmaceutical Division, 35,868. Macclesfield, Cheshire, England.

Rotary tumbler

CHILTERN, SMITH BIOLAB LTD., Auckland, New Zealand.

Screw-cap glass tube

PYREX IWAKIGLASS, IWAKI GLASS Co, LTD., Chiyda-Ku Tokyo, Japan.

Sodium dihydrogen phosphate

$NaH_2PO_4.H_2O$ 0.1M M=137.99/mol. Guarantee grade, MERCK Art 6346, E. Merck, Frankfurt, Germany.

Sodium hydroxide solution

C(NaOH)=1 mol/L (1N) Volumetric solution MERCK Art 9137, E. Merck, Frankfurt, Germany.

Spectra/Por equilibrium Dialyser

Spectra/Por, SPECTRUM MEDICAL IND., INC, Los Angeles, California, U.S.A.

Stir plate

NOOVA II SYBRON. Thermolyne, Dubuque, Iowa, U.S.A.

Tetramethylammonium Hydroxide solid

Pentahydrate Formula Wt 181.2, SIGMA Chemical Company St. Louis, Mo, U.S.A.

Thymol Crystals

$C_{10}H_{14O}$ M=150.22g/mol Extra pure, MERCK Art 8167, E. Merck, Frankfurt, Germany.

Ultrasonic cleaner

BRANSON SmithKline, BRANSON CLEANING EQT. Company, Woburn, MA, U.S.A.

Water bath

EDELSTAL Rost frei, MEMERT-GmbH, Postfach, Schwabach, Germany.